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## USE OF ALTERNATING TEMPERATURES IN THE GERMINATION OF SEEDS<sup>1</sup>

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### INTRODUCTION

The helpful use of daily alternations of temperatures in the germination of certain seeds grew out of observed differences in germination in light as compared with that in darkness, and the resulting controversy as to whether the favoring effect of light was due to the heat rays or was photochemical in nature.

Cieslar (3)<sup>2</sup> in 1883 secured better germination of *Poa nemoralis* in yellow (therefore warm) light than in white light, although violet (therefore cold) light gave no better germination than was secured in darkness. Nickholz (19), working with *Poa pratensis*, later reported similar results which indicated that the heat rays were more effective in germination than the light rays. While this conclusion has not proved to be true with all light-sensitive seeds, it has with some kinds; and the investigations which followed Cieslar's work led to the definite policy of germinating many kinds of seeds with a regular daily alternation of temperature. Usually about 20° C. has been used as the lower temperature and about 30° as the higher temperature, the higher temperature being maintained for from four to eight hours each day. Many other alternations have been used experimentally with good results.

The year following Cieslar's publication Von Liebenberg (16) showed that *Poa pratensis* germinated better when kept at 20° C. for 19 hours and at 28° for five hours of each day than at either 20° or 28°, constantly maintained, and that this alternation of temperatures took the place of exposure of the seed to light. Since then others (1, 2, 6, 7, 8, 9, 11, 15, 17, 18, 19, 20, 21) have shown a favorable effect of temperature alternations upon the germination of many kinds of seeds. Usually a daily alternation has been used. Heinrich (13), however, showed that a very beneficial effect was produced in some cases by a definite temperature range once in five days. Lehman (15) argued that a similar favorable effect was produced by temperature changes without definite or daily alternations, and Gassner (6) showed that only a few hours at a low temperature followed by a single change to a higher temperature induced maximal germination of seeds of *Paspalum dilatatum*, which would not germinate well without previous exposure to the low temperature.

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<sup>2</sup>Reference is made by number (italic) to "Literature cited," p. 331-332.

Haberlandt (10) and Eidam (5) have suggested temperature effects upon the water intake of seeds as a possible cause of the favorable effect of temperature alternations upon germination. This hypothesis seems hardly tenable, since certainly some and probably most seeds whose germination is favored by temperature alternations take up at any temperature more water than the minimum required for germination. Von Liebenberg (16) suggested that the reserve materials made available at any given temperature were wholly or largely used up in respiration, but that a slight surplus, becoming soluble at higher temperatures, is available for growth when the temperature is lowered with consequent reduction in the intensity of respiration. Gassner (7) considered the effect of temperature alternations upon the germination of *Chloris ciliata* to be entirely a matter of oxygen relations. In line with Gassner's conclusion Vanha (21) called attention to the fact that differences in temperature between the different parts of the seed, the germinating bed, and the outer air following a sudden temperature change cause different gas densities which may set up lively gas movements leading to removal of CO<sub>2</sub> and renewal of oxygen—conditions favorable to increased respiration and probably to germination.

Probably none of these hypotheses can furnish a complete explanation of the effect of temperature alternations, but they point the way for an attack upon the problem. A detailed study of the respiration and internal changes of the seeds at different temperatures would probably help in its solution. The respiratory quotient of dormant apple seeds decreases with decreasing temperature, and vice versa. If this is true of dormant embryos generally, there may be at low temperatures an accumulation and actual metabolism of oxygen in a form which becomes immediately available for the inception of growth processes when the seeds are placed at the higher temperature. This hypothesis is in some respects the converse of that of Von Liebenberg (16), but it does not stand in opposition to that of Vanha (21) or to that of Gassner (7).

The purpose of the present paper is not to discuss the possible explanation of the effects of temperature alternations upon germination but merely to show somewhat more in detail the nature and extent of such effects.

#### EXPERIMENTAL WORK

Several years ago seeds of carrot (*Daucus carota* L.), celery (*Celerio graveolens* (L.) Britton), parsley (*Petroselinum hortense* Hoffm.), and parsnip (*Pastinaca sativa* L.) were tested for germination at 15°, 20°, 25°, 30°, and 35° C., and with every possible alteration between these temperatures in which the higher temperature was maintained 7½ hours and the lower temperature 16½ hours of each day—a total of 5 constant temperatures and 10 temperature alternations. The temperature alternations were accomplished by transferring the trays with their load of seeds from one germination chamber to another and back again each day.

Under nearly all of these 15 temperature conditions, germination tests of seeds of the following grasses were made: Timothy (*Phleum pratense* L.), awnless brome grass (*Bromus inermis* Leyss.), perennial rye grass (*Lolium perenne* L.), Italian rye grass (*Lolium multiflorum* Lam.), meadow-fescue (*Festuca elatior* L.), orchard grass (*Dactylis glomerata* L.), redtop, (*Agrostis polustris* Huds.), Kentucky bluegrass (*Poa pratensis* L.), and Bermuda grass (*Cyniopsis dactylon* (L.) Kuntze).

In these germination tests it was not possible with the equipment available to control the temperatures absolutely, but except for 15° and 20° C. the fluctuations were very slight. Even with these lower temperatures the control was sufficiently accurate to give reasonably reliable results, as will appear later.

Seeds of celery and redtop were tested on top of four thicknesses of moistened blotting paper. Kentucky bluegrass and Bermuda grass seed were tested with the Jacobson apparatus (4, p. 33). The other kinds were tested between moistened blotting papers, two thicknesses below and two above.

The following year a study was made of the actual progress of temperature changes in the interior of the germinating chambers in a variety of temperature alternations, and these temperature changes, with the resulting effects upon the germination of a number of kinds of seeds, were compared with data obtained from field experiments.

About two years later a number of kinds of flower seeds were tested at very accurately maintained constant temperature from 15° to 30° C. with a large number of temperature alternations. Some kinds were tested also in an ice box. The kinds studied were balsam (*Impatiens balsamina* L.), California poppy (*Eschscholtzia californica* Cham.), candy-tuft (*Iberis amara* L.), cosmos (*Cosmos bipinnatus* Cav.), belvedere (*Kochia scoparia* (L.) Schrad.), larkspur (*Delphinium ajacis* L.), marigold (*Calendula officinalis* L.), mignonette (*Reseda odorata* L.), nasturtium (*Tropaeolum majus* L. and *T. minus* L.), pansy (*Viola tricolor* L.), petunia (*Petunia hybrida* Hort.), Chinese pink (*Dianthus chinensis* L.), poppy (*Papaver* spp.), portulaca (*Portulaca grandiflora spelodens* Hort.), snapdragon (*Antirrhinum majus* L.), sweet pea (*Lathyrus odoratus* L.), and zinnia (*Zinnia elegans* Jacq.). The larger seeds were tested between wet blotters or wet cotton flannel and the smaller ones on top of wet blotters.

More recently a careful study was made of the germination of Johnson grass seed (*Holcus halepensis* L.) under accurately controlled temperature conditions, moistened blotting paper disks in 100-mm. Petri dishes being used as a germinating bed.

In the germination tests of each series duplicates of 100 or 200 seeds each were used. The seeds used were fully after-ripened except as indicated later in certain tests of Johnson grass seed. The tests whose results are presented in this paper are typical examples of a large number of tests of the same kinds of seed.

The results of the first and third series of tests will be presented briefly and then the results of the tests of Johnson grass seed will be given more in detail, reserving the study of the alternations and comparison with field data until last. On account of the nature and complexity of the data dealt with, the results can in general be presented to best advantage in graphic rather than in tabular form.

## RESULTS OF THE EXPERIMENTS

### I. SEEDS GERMINATING AT CONSTANT TEMPERATURES

Seeds of timothy, awnless brome grass, both species of rye grass, meadow fescue, carrot, and parsley and all of the flower seeds germinate nearly or quite as rapidly and completely at favorable constant temperatures as with any alternation of temperatures. The most

favorable constant temperature for the different species varied from 15° C. for larkspur and poppy seeds to 22.5° or 25° for petunia seed. Some kinds of seed germinated equally well with a wide range of temperatures, while for others there was a very definite temperature optimum. For instance, larkspur seed germinated much better at 15°, which represents its optimum, than at 17.5°, while belvedere seed germinated with

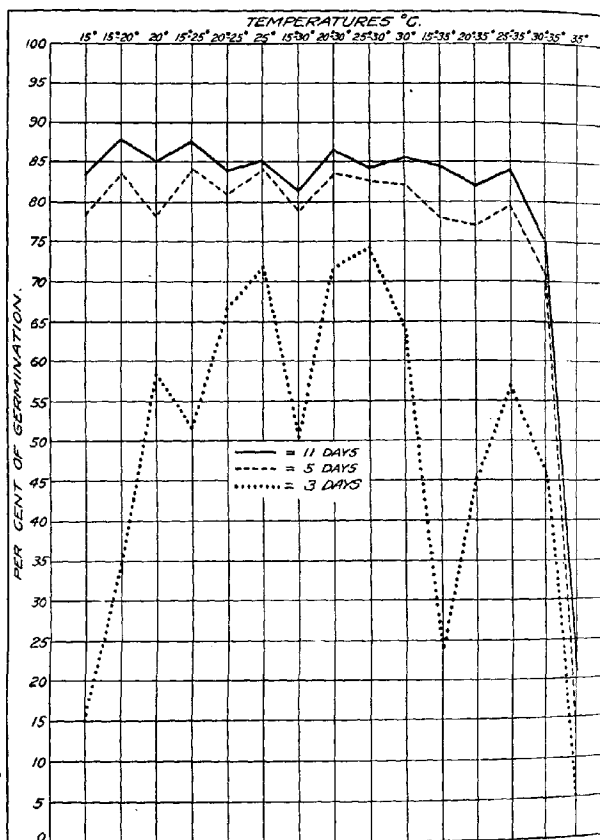


FIG. 1.—Average rates and percentages of germination of two lots of carrot seed under 15 temperature conditions.

equal completeness and with very little difference in rapidity at any temperature from 15° to 30°.

Figure 1, representing the average rates and percentages of germination of two lots of carrot seeds under 15 temperature conditions, illustrates the behavior of those seeds which germinated well at constant temperature and were tolerant of a wide range of temperatures. The figure shows a retarding effect of very cool or very warm temperatures and of alternations of temperature between extremes which are widely

separated from each other. But the differences observed in the early days of the test were evened up toward the end, except with the warm temperature alternation  $30^{\circ}$  to  $35^{\circ}$  C. and at  $35^{\circ}$  constant.

## 2. SEEDS REQUIRING TEMPERATURE ALTERNATIONS

Seeds of redtop, orchard grass, Kentucky bluegrass, Bermuda grass, parsnip, celery, and Johnson grass required an alternation of temperatures for best germination. Of these, redtop comes nearest to being a constant-temperature germinator and Bermuda grass stands at the other end of the series.

### REDTOP

Figure 2 shows the average percentages and rates of germination of three samples of redtop seed. Alternations between extremes  $10^{\circ}$  C. apart ( $20^{\circ}$  to  $30^{\circ}$  and  $25^{\circ}$  to  $35^{\circ}$ ) gave the highest results, with  $5^{\circ}$  and  $15^{\circ}$  alternations only slightly lower but still somewhat ahead of the constant temperatures. As with the constant-temperature germinators, so here in the tests with redtop, constant temperatures below or very much above the optimum and alternations  $15^{\circ}$  wide ( $20^{\circ}$  to  $35^{\circ}$ ) retarded germination in the early days of the test. At the end of the first three days the influence

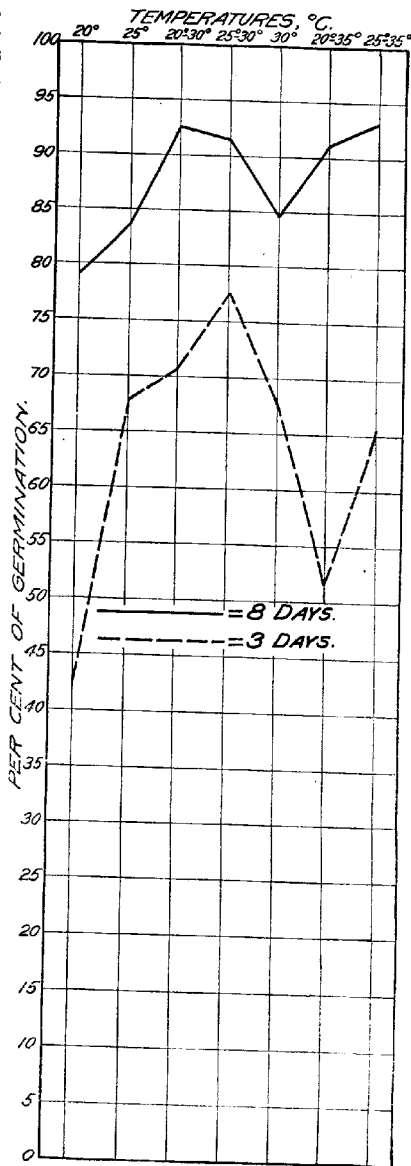


FIG. 2.—Average rates and percentages of germination of three lots of redtop seed under seven temperature conditions.

of the actual mean temperature seems to predominate, except with the wide alternation  $20^{\circ}$  to  $35^{\circ}$ , over the influence of alternations as contrasted with constant temperatures; and it is only in the later days

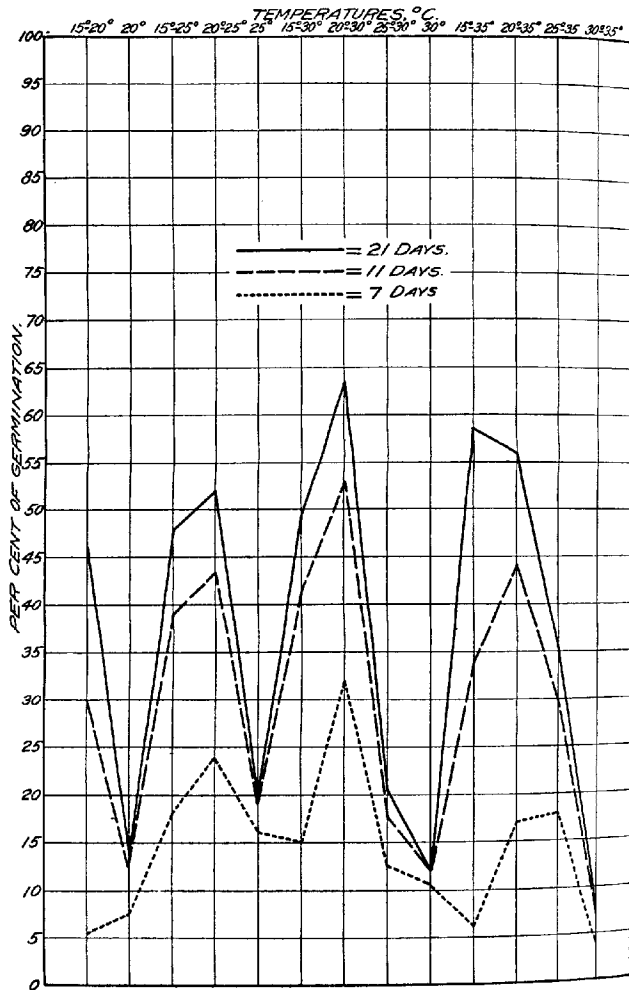


FIG. 3.—Average rates and percentages of germination of three lots of Kentucky blue-grass seed under 13 temperature conditions.

of the tests that some of the alternations prove their superiority. From the standpoint of rapidity of germination, alternations with  $30^{\circ}$  are preferable to alternations with  $35^{\circ}$  as the higher temperature.

## KENTUCKY BLUEGRASS

Figure 3 shows the average percentages and rates of germination of three lots of Kentucky bluegrass seed. Here the favorable effect of temperature alternations is strikingly evident and increases with the progress of the test. All of the low points in the graphs, except for the earliest count (seven days), represent germination at constant temperatures or with narrow ( $5^{\circ}\text{C.}$ ) alternations. A narrow alternation was more effective in a low temperature range ( $15^{\circ}$  to  $20^{\circ}$  and  $20^{\circ}$  to  $25^{\circ}$ ) than in a higher temperature range ( $25^{\circ}$  to  $30^{\circ}$ ). This has been found to be true also with other seeds requiring temperature alternations for best germination. An alternation of  $15^{\circ}$  retarded germination in the early days of the test. The alternations  $25^{\circ}$  to  $35^{\circ}$  and  $30^{\circ}$  to  $35^{\circ}$  were too warm for good results.

It should be emphasized here that the beneficial effect of temperature alternations can not in any way be referred to a constant temperature equivalent to the mean temperature of the alternation or to the extreme temperatures of the alternation. Thus, temperature alternations with  $20^{\circ}\text{C.}$  as either upper or lower extreme gave much better results than  $20^{\circ}$  constant, and any alternation gave better results than either of the extremes of that alternation constantly maintained. Again, the germination with the alternations  $15^{\circ}$  to  $30^{\circ}$  and  $20^{\circ}$  to  $35^{\circ}$  was approximately three times as great as at their constant temperature equivalents,  $20^{\circ}$  and  $25^{\circ}$ .

At the same time it is possible to see an effect of mean daily temperature supplementing the more important effect of the temperature changes as such. Since the higher temperature in each alternation was maintained for approximately one-third of each day the mean temperature of each alternation can be represented by adding to the lower temperature one-third of the difference between that and the higher temperature. Table I shows the mean temperatures of the alternations which gave fairly good results, together with the average total percentages of germination. The germination increased regularly with increase in the mean temperature of the alternation up to  $23^{\circ}\text{C.}$  and fell rather abruptly after this mean. There was a similar correspondence between mean temperatures and the germination of Johnson grass seed, but not with any of the other seeds which required alternations of temperatures for best germination.

TABLE I.—Temperature alternations, corresponding mean temperatures, and average percentages of germination produced in three lots of Kentucky bluegrass seed

Temperature alternation.	Approximate mean temperature.	Average percentage of germination.	Temperature alternation.	Approximate mean temperature.	Average percentage of germination.
$^{\circ}\text{C.}$	$^{\circ}\text{C.}$		$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	
15 to 20.....	16	46	15 to 35.....	22	38
15 to 25.....	18	48	20 to 30.....	23	63
15 to 30.....	20	49	20 to 35.....	25	56
20 to 25.....	21	52	25 to 35.....	28	35

Kentucky bluegrass is one of the kinds of seed with which Hartleb and Stulzer (12) secured much better germination at  $30^{\circ}\text{C.}$  than with an alternation between  $20^{\circ}$  and  $30^{\circ}$ . The results here reported, as well as the results of several other investigators, show that Hartleb and Stulzer's results were unreliable and their conclusions false.



The temperature alternation giving best results in these tests ( $20^{\circ}$  to  $30^{\circ}$  C.) is almost identical in mean temperature with that ( $21^{\circ}$  for 8 hours and  $28^{\circ}$  for 16 hours) which Vanha (21) in his careful and thorough investigation found most advantageous for the germination of this species. Brown (1) also recommended an alternation between  $20^{\circ}$  and  $30^{\circ}$  for the germination of seeds of this species.

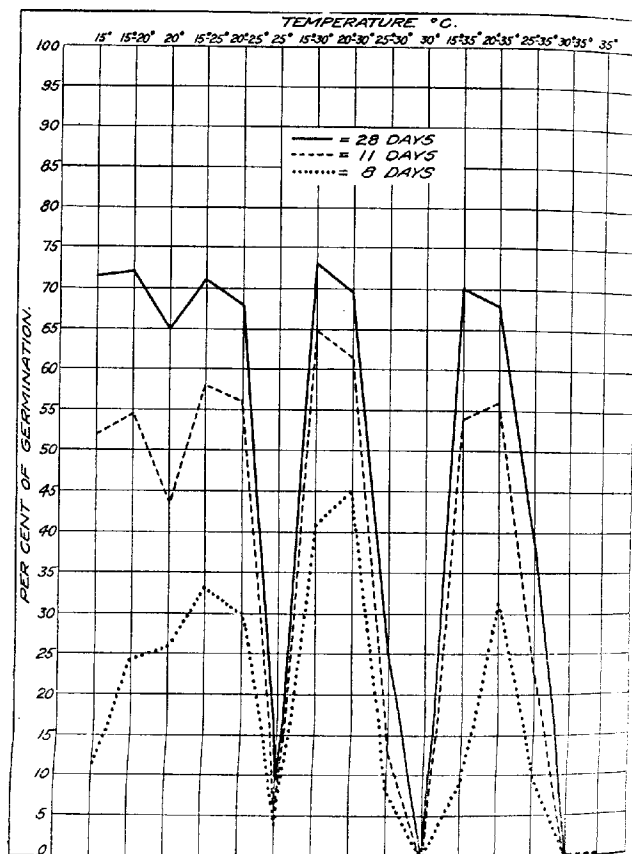


FIG. 4.—Average rates and percentages of germination of three lots of celeryseed under 15 temperature conditions.

#### CELERY

Figure 4 shows that celery seed germinated almost as completely at nearly constant low temperatures as with temperature alternations, though it germinated more slowly. However, as the germination temperature increased a relatively wide alternation ( $10^{\circ}$  C.) became imperative and still wider alternation ( $15^{\circ}$  or  $20^{\circ}$ ) did no harm. As with Ken-

tucky bluegrass, the favorable effect of the alternations is not referable to the effect of extreme or mean temperatures but is the result of the changes in temperature. In several other tests of celery seed with better control of the lower temperatures, germination was much poorer at 20° and with the alternation 20° to 25°, in comparison with other alternations, than in the tests represented in Figure 4. No other tests were made at 15° or with the alternation 15° to 20°.

The slight fluctuations in the temperature of the cool germinating chambers were no doubt partly responsible for the high percentages of germination at low temperatures; but they certainly were not sufficient to explain the results entirely if the seeds required as wide alternations at low temperatures as at high temperatures and if no other significant factor was involved. Additional tests made at another time indicate, however, that other factors may be involved. Ninety-six simultaneous tests of celery seed from a single unusually sensitive lot were made in different parts of a single chamber, which was heated from below by a gas burner during the several hours of each forenoon and cooled each afternoon by a stream of cold water in the top of the water jacket around the chamber. The upper and lower temperatures were controlled by thermo regulators, and the lower temperature was held for about 15 hours of each day. The seeds in the more rapidly cooled parts of the chamber germinated much more rapidly and completely than those in other parts of the chamber, though the extreme upper and lower temperatures reached were very nearly the same in all parts of the chamber. The actual range in percentage of germination in different parts of the chamber was from 0 to 32 per cent in 7 days, from 0 to 58 per cent in 9 days, from 11 to 81 per cent in 11 days, from 41 to 91 per cent in 14 days, and from 53 to 95 per cent in 21 days. After 21 days the seeds which had occupied the least favorable positions and had germinated most poorly were put in the most favorable positions, with the result that their germination soon increased to equal that of the seeds originally in the more favorable positions.

Similarly, when the celery seed was daily transferred between two chambers constantly maintained at different temperatures and the cool chamber was cooled by a block of ice above the water jacket, as was done in the tests represented in Figure 4, the seeds germinated somewhat better if placed in the top of the cool chamber than if placed lower in the chamber where the temperature changes were less abrupt.

In the tests discussed in the two preceding paragraphs the temperatures in the positions in which the seeds germinated best were almost always somewhat lower than in the other parts of the chambers. The differences in temperature were not, however, commensurate with the corresponding differences in germination. In the more rapidly cooled and constantly somewhat cooler parts of the cool chambers convection currents of air must have been more lively than in other parts, and this may have led to a significantly better renewal of oxygen in immediate contact with the seeds, as suggested by Vanha (21). Also there was a constant tendency in the cooler parts of the chambers for water from the air which had become saturated in the warmer parts of the chambers to condense upon the surface of the seeds. This condensation water would presumably be saturated with oxygen, which would be immediately available for the use of the seeds unless kept out by seed coats relatively impermeable to oxygen. While there is no direct evidence that oxygen relations really are important here this possibility must be admitted.

## BERMUDA GRASS

The favorable effect of temperature alternations is strikingly evident in Figure 5. A very narrow alternation ( $5^{\circ}$  C.) is almost without effect, and a  $10^{\circ}$  alternation is much less effective than a  $15^{\circ}$  or  $20^{\circ}$  alternation.

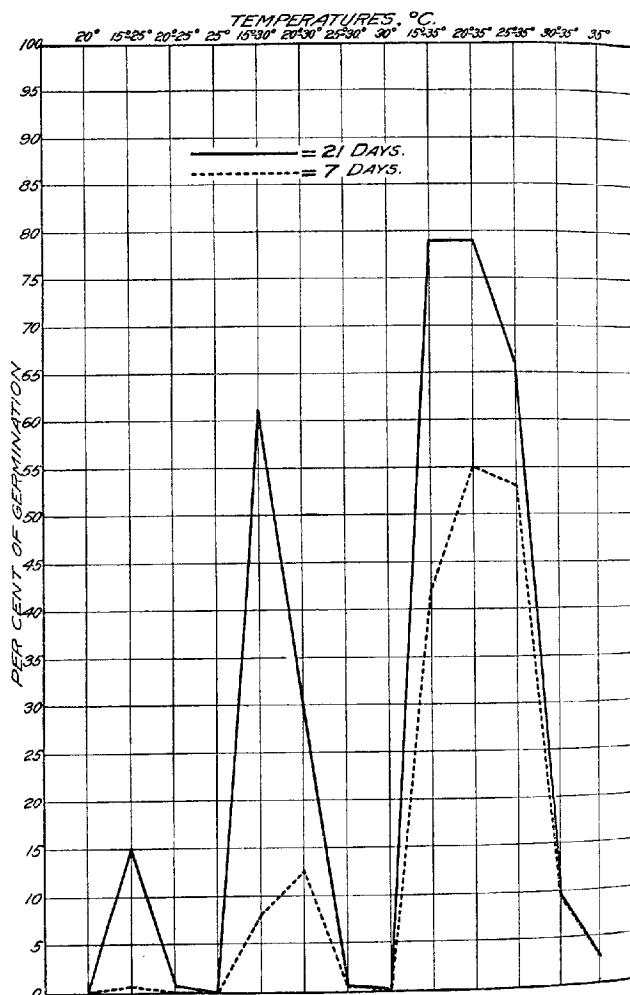


FIG. 5.—Average rates and percentages of germination of three lots of Bermuda grass seed under 13 temperature conditions.

Bermuda grass seed germinates at high temperature, its germination being better at  $35^{\circ}$  and with the  $5^{\circ}$  alternation below  $35^{\circ}$  than at lower constant temperatures or with  $5^{\circ}$  alternations in a lower temperature

range. In view of the temperature effects here manifested and the work with Johnson grass seed discussed later in this paper, it is unfortunate that experimental germination tests of Bermuda grass seed were not also made with still warmer temperatures, both constant and in alternation.

#### ORCHARD GRASS AND PARSNIP

These two kinds of seed required an alternation of temperatures for best germination. They were considerably more sensitive to temperature conditions, especially to the difference between constant temperatures and temperature alternations, than redtop seed (fig. 2.), but somewhat less sensitive than Kentucky bluegrass (fig. 3) or celery seed (fig. 4). With each of these two species every alternation of temperatures except  $25^{\circ}$  C. to  $30^{\circ}$  and  $30^{\circ}$  to  $35^{\circ}$  gave better results than any constant temperature.

#### REVERSE ALTERNATIONS

Parsnip and celery seed were tested with the alternations  $25^{\circ}$  C. for  $16\frac{1}{2}$  hours and  $20^{\circ}$  for  $7\frac{1}{2}$  hours each day in comparison with the alternation  $20^{\circ}$  for  $16\frac{1}{2}$  hours and  $25^{\circ}$   $7\frac{1}{2}$  hours each day and with the similar alternations  $30^{\circ}$  to  $20^{\circ}$  and  $20^{\circ}$  to  $30^{\circ}$ . The alternations in which the cool temperature was maintained for the longer time gave uniformly better germination than the reverse of these alternations. The differences were frequently large, but with some lots of seed they were insignificant.

#### JOHNSON GRASS

It has already been pointed out (11): (1) That newly matured Johnson grass seed is characterized by a dormancy which is not resident in the embryo but is imposed by coat structures and which is not entirely removed until after several months of dry storage, and (2) that both during and after the process of after-ripening the seeds of this species germinate better at a high temperature and with temperature alternations than at lower or constant temperatures. In the earlier paper (11) an alternation between  $25^{\circ}$  and  $40^{\circ}$  C. was given as the optimum temperature condition for the germination of this kind of seed, but later work has shown that the still higher temperature alternation  $30^{\circ}$  to  $45^{\circ}$  gives still better results.

Figure 6 shows the average rates and percentages of germination of six lots of fully after-ripened Johnson grass seed under 12 temperature conditions, the higher temperature in each alternation being maintained from six to eight hours each day.

The accelerating effect of warm temperatures is shown by the fact that, in contrast to all the kinds of seed hitherto discussed, Johnson grass seed germinated more rapidly in the early days of the germination test at constant temperatures than with the temperature alternations corresponding to a lower mean temperature, but just about the same as with temperature alternations corresponding to the same mean temperature. The high points in the graph for germination in two days all correspond to constant temperatures, but the points in this graph showing germination with the temperature alternations  $20^{\circ}$  to  $35^{\circ}$  C.,  $25^{\circ}$  to  $40^{\circ}$ , and  $30^{\circ}$  to  $45^{\circ}$ , fall on almost exactly the same levels, respectively, as those for the approximately equivalent constant temperatures  $25^{\circ}$ ,  $30^{\circ}$ , and  $35^{\circ}$ .

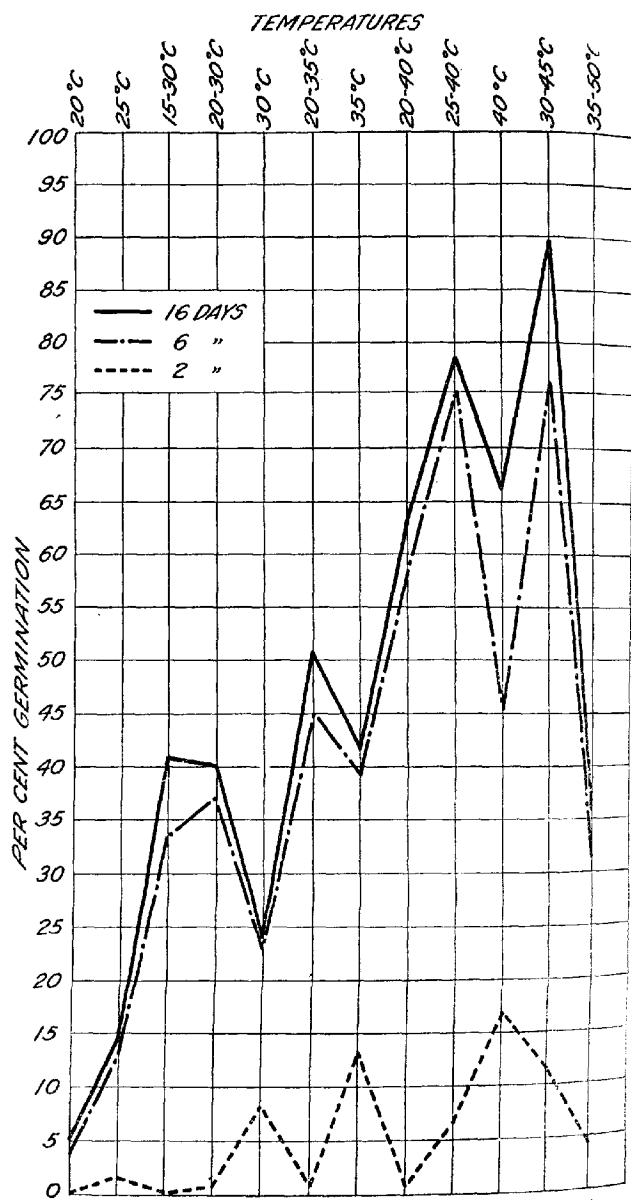


FIG. 6.—Average rates and percentages of germination of six lots of Johnson grass seed under 12 temperature conditions.

As germination advanced the alternations proved more advantageous and the shape of the graphs representing germination became reversed, with the low points falling on the lines for constant temperatures.

The alternation 30° to 45° C. gave best results, and 25° to 40° next best.<sup>1</sup> With the alternation 30° to 45° germination was more rapid at first, progressed at a more nearly uniform rate, and was more active in the last 10 days of the germination test than with the alternation 25° to 40°. With the latter alternation nearly all of the germination occurred between the second and the sixth days.

As with Kentucky bluegrass seed (see p. 301), the effect of the mean temperature of the alternations supplements in a definite manner the more important effect of the temperature changes as such. The alternation 20° to 30° C. evidently is not wide enough to produce the desired results. Table II compares the other alternations used and the percentages of germination on the basis of the approximate mean temperatures of the alternations. The percentage of germination rose regularly with rising mean temperature up to 34° and then fell abruptly.

At 40° C. constant, germination was spread over an even longer period than with the alternation 30° to 45°. It is important to remark here that while some seeds produced normal seedlings at this high temperature (40°) many at this temperature and a few at a constant temperature of 35° germinated only sufficiently to spread the heavy scales which inclose the caryopses by the slight elongation of radicle or coleoptile, after which growth ceased. These seeds were counted as germinated. When such seeds, after growth had stopped, were placed at a cooler temperature, either constantly or in alternations, normal growth ensued. One is reminded here of Kidd & West's work (14) in which a variety of agents induced growth in dormant white mustard seeds when used in concentrations or degrees just falling short of causing serious injury to the seed. According to this conception heat might here be considered as a stimulus to germination; and the advantage of an alternation between temperatures, the warmer of which lies either near the upper limit of endurance of a given seed or above the optimum for its germination and the subsequent growth of the seedling, might lie in the fact that the seed is given the temporary and recurring advantage of an elevation of temperature without being subjected to the harmful effects of long-continued exposure to a high temperature. In this connection it should be remembered also that in the case of a temperature alternation the actual temperature of the seeds probably never quite reached the temperature shown on the thermometer in the warmer germinating chamber and that the rise and fall in the temperatures of the seeds were relatively slow and gradual.

TABLE II.—Temperature alternations, corresponding mean temperatures, and average percentages of germination produced in six lots of Johnson grass seed

Temperature alternation.	Approximate mean temperature.	Average percentage of germination.	Temperature alternation.	Approximate mean temperature.	Average percentage of germination.
°C.	°C.		°C.	°C.	
15 to 30.....	19	41	25 to 40.....	29	78
20 to 35.....	24	51	30 to 45.....	34	88
25 to 40.....	25	63	35 to 50.....	39	36

<sup>1</sup> Auxiliary viability tests of the seeds remaining ungerminated with the alternation 30° to 45° C. were made by removing the scales and pricking according to a method previously described (11). They showed by the seeds originally put to germinate an average viability of 93 per cent, which is only 4 per cent greater than the germination without special treatment.

With the extremely warm temperature alternation, 35° to 50° C., the percentage of germination was low and the majority of the seedlings failed to make normal growth.

Concurrent germination tests of the six lots of seeds whose germination is represented in Figure 6 were made also at 15°, 45°, and 50° C. and with the temperature alternation 40° to 55°. Less than 1 per cent germinated at 15° C., and these made but little growth. At the higher temperatures none germinated in six days. These seeds were then given the temperature alternation 25° to 40° C. for seven days, followed by viability tests<sup>4</sup> with the alternation 30° to 45° C. Viability tests showed that all but a fraction of 1 per cent had been killed by six days' exposure to 50° C. or the alternation 40° to 55°. A temperature of 45° C. for six days had killed more than half of the seeds of some of the lots, but only a few of the seeds of other lots. The seedlings produced were relatively weak.

#### Differences between individual lots of seed

Different lots of Johnson grass seed vary greatly in the readiness with which they germinate, and it has been found that poorly germinating lots are more sensitive to temperature conditions than lots which germinate more readily. This is illustrated in Figure 7 which represents the total germination in 16 days of two of the lots included in the averages for Figure 6. No. 8599 was grown at Arlington Farm, Va., and gave a fairly ready germination. The San Antonio lot was collected from wild plants near San Antonio, Tex., and gave poor germination. While the general shape of the two graphs in Figure 7 is the same, favorable alternations and especially the best alternation, 30° to 45° C. showed greater advantage over constant temperatures and less favorable alternations in the case of the San Antonio lot than in the case of the other lot. Viability tests<sup>5</sup> showed 94 per cent of the San Antonio lot and 95 per cent of the other lot to be alive and capable of producing vigorous seedlings.

#### Effect of removal of the scales

The effect of removal of the scales (glumes, or hulls) upon the germination of Johnson grass caryopses has already been pointed out (11). The effect is illustrated in Figure 8, which shows the average percentage of germination in 13 days of two lots of seed intact and with the scales removed. The increase in germination as a result of removing the scales is especially noteworthy at the warm constant temperatures 30°, 35°, and 40° C.

In the preceding section it was shown that readily germinating lots were less sensitive to temperature conditions than lots which germinated poorly. Increasing the readiness with which a given lot germinates and at the same time diminishing its sensitiveness to temperature conditions by removal of the scales merely constitutes a special case of the previous rule. The rapidity of germination also is greatly increased by removal of the scales.

<sup>4</sup> See footnote on page 307.

<sup>5</sup> See footnote on page 307.

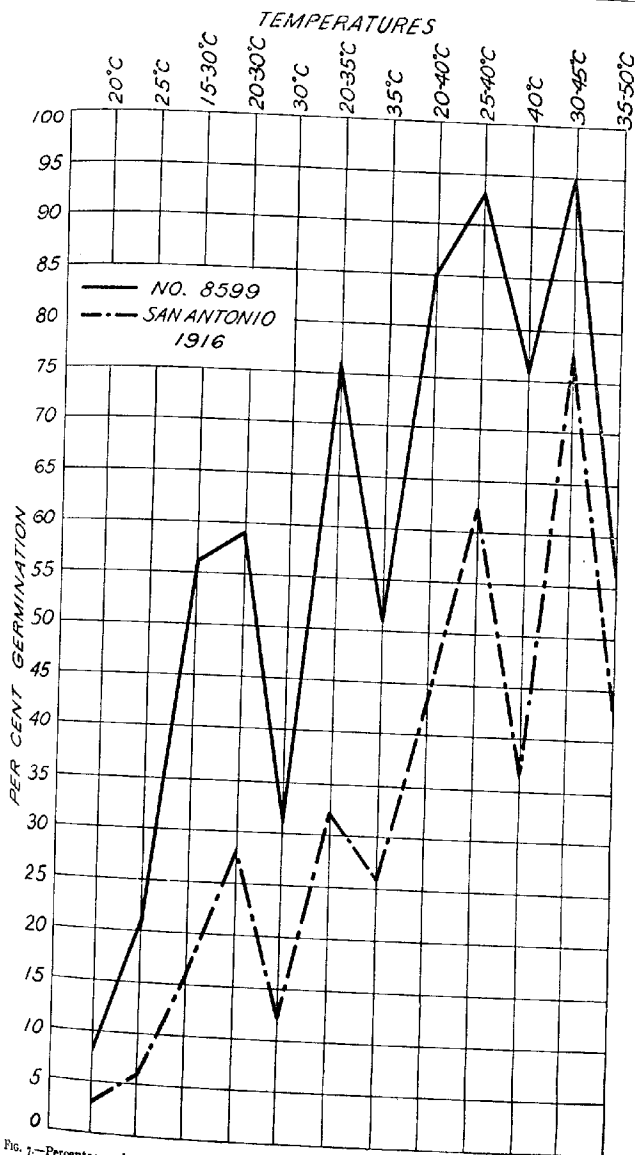


FIG. 7.—Percentages of germination in 16 days of two lots of Johnson grass seed under 12 temperature conditions.



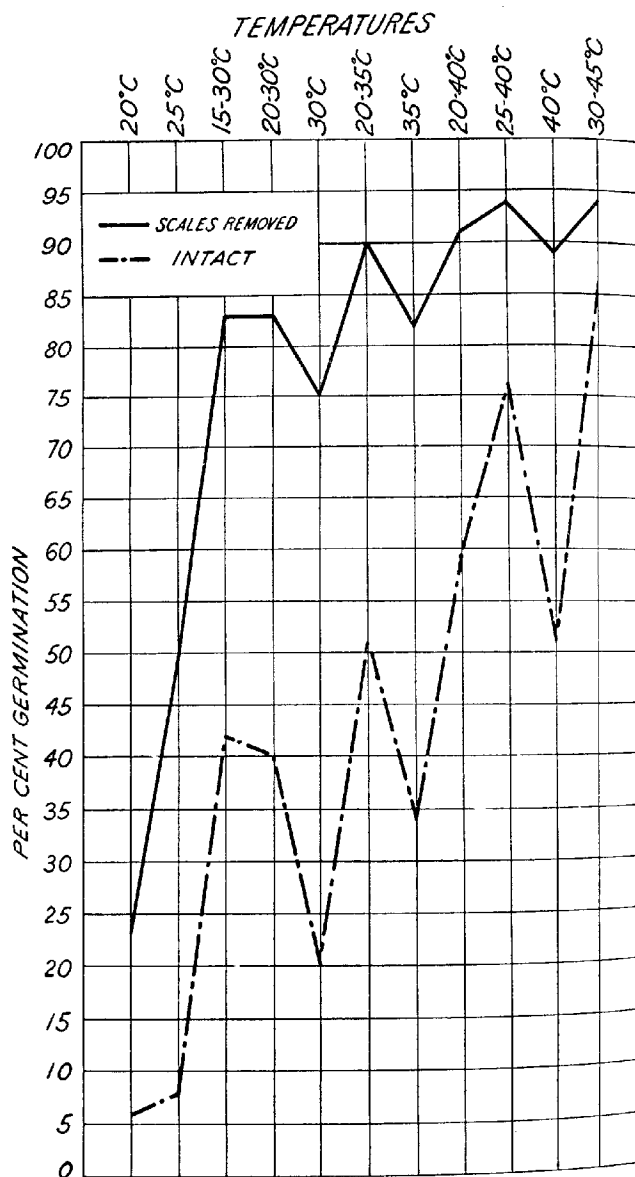


FIG. 8.—Average percentages of germination of two lots of Johnson grass seed, intact and with scales removed.

*Effect of various lengths of time at the warmer temperature of an alternation*

Six lots of seed were germinated with temperature alternations of  $25^{\circ}$  to  $40^{\circ}$  C. and  $30^{\circ}$  to  $45^{\circ}$  C., in which the seeds were in the warmer chamber two, four, six, and eight hours each day. Figure 9 shows the average germination. The numerals in the position of exponents to the temperature conditions at the top of the figure indicate the number of hours at the warmer temperatures. This figure illustrates again the fact noted in connection with Figure 6, that germination is more rapid during the first few days, proceeds at a more nearly uniform rate, continues for a longer time, and reaches a higher total percentage with the alternations  $30^{\circ}$  to  $45^{\circ}$  than with the alternations  $25^{\circ}$  to  $40^{\circ}$ . Eight hours daily at  $45^{\circ}$ , however, reduced the rapidity of germination in the first two days below that with the alternations  $25^{\circ}$  to  $40^{\circ}$ . The rapidity of germination with the alternations  $25^{\circ}$  to  $40^{\circ}$  increased regularly as the length of time in the warmer germinating chambers increased.

Differences in response of individual lots of seeds to these eight temperature conditions are shown in Figure 10 which gives the percentages of germination of three lots of seed in 13 days.

No. 8604 was a readily germinating lot composed of seed about 10 months old. The germination of this lot was, within the limits of experimental error, identical under all eight temperature conditions.

No. 1417 was about 2 years old. It germinated much better with the alternations  $30^{\circ}$  to  $45^{\circ}$  C. than with the alternations  $25^{\circ}$  to  $40^{\circ}$ , but showed little difference according to the length of time in the warmer chamber.

The lot designated "San Antonio, 1917," was harvested from wild plants near San Antonio, Tex., a few weeks before the tests were made and had not after-ripened. In contrast to No. 1417, this lot germinated better with the alternations  $25^{\circ}$  to  $40^{\circ}$  C. than with those  $30^{\circ}$  to  $45^{\circ}$ ; with both sets of alternations the percentage of germination decreased with increase in the length of time in the warmer chamber. At the end of 13 days this lot of seed was still germinating at the rate of about  $\frac{1}{2}$  per cent per day, more rapidly with the alternations  $30^{\circ}$  to  $45^{\circ}$  C. than with the alternations  $25^{\circ}$  to  $40^{\circ}$ , so that the germination with the two sets of alternations would have become more or less equalized with longer germination period. Whether the difference between this lot and No. 1417 is characteristic of differences between old seed and fresh seed not after-ripened is not known. Seed from the same locality as the San Antonio lot, collected one year earlier and tested at the same time, germinated better with all four of the alternations between  $30^{\circ}$  and  $45^{\circ}$  C. than with the alternations between  $25^{\circ}$  and  $40^{\circ}$ , in this respect resembling No. 1417. Furthermore, it is known that the germination temperatures of some seeds change with the progress of after-ripening. At any rate the differences obtained here illustrate the apparent impossibility of finding any single temperature condition which will be the optimum for every individual lot of seed, regardless of its peculiarities or its previous history.

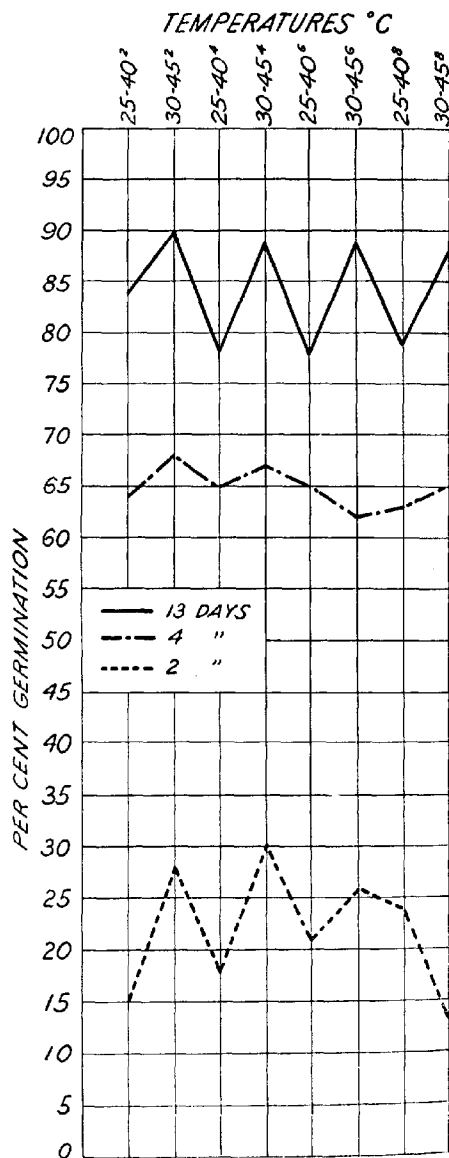


FIG. 9.—Percentages of germination of two after-ripened lots and one fresh lot of Johnson grass seed in 13 days with eight temperature alternations.

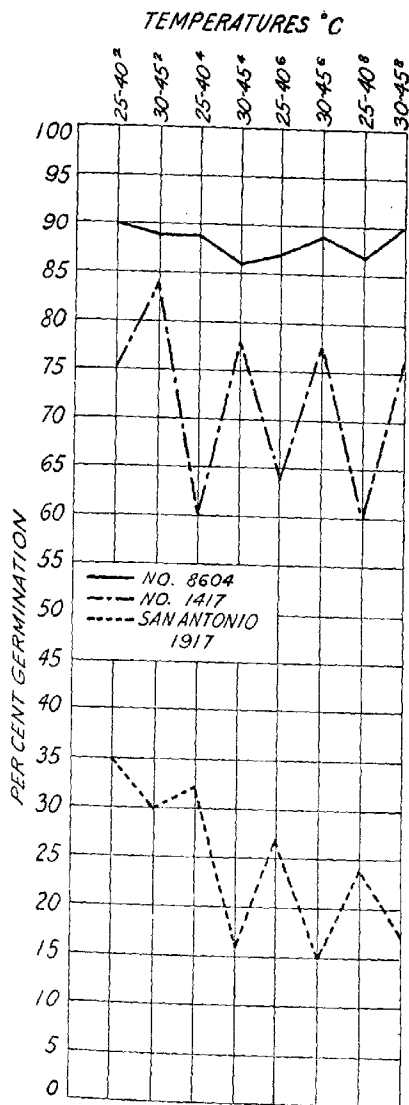


FIG. 10.—Average rates and percentages of germination of six lots of Johnson grass seed with alternations in which the seeds were in the warmer chambers for different lengths of time.

### 3. PROGRESS OF TEMPERATURE CHANGES IN RELATION TO GERMINATION: COMPARISON WITH FIELD TESTS

From December, 1911, to May, 1912, germination tests were made with about 30 kinds of seed, mentioned on p. 322, under 11 different temperature alternations.

Kentucky bluegrass seed was tested in the Jacobson apparatus (4); and the other kinds were placed between blotters or on top of blotters, according to the size of the seeds. Each chamber was constantly filled

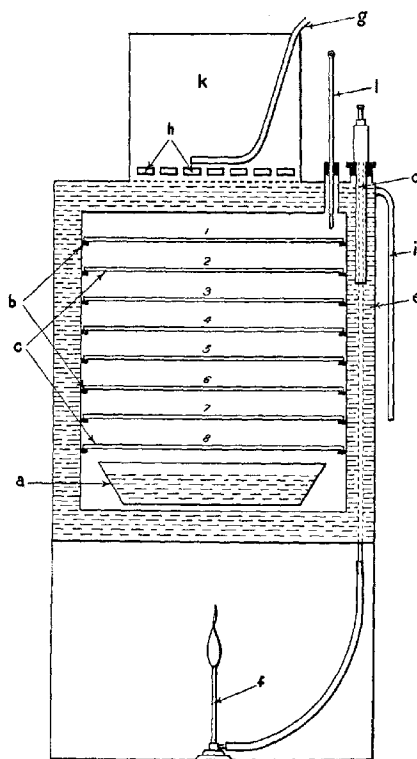


FIG. 11.—Median section of a germinating chamber: (a), pan of water; (b), ledges to support seed trays; (c), seed trays (numbered from 1 to 8); (d), thermo-regulator; (e), water jacket; (f), burner; (g), cold-water pipe; (h), perforated copper plate; (i), overflow pipe; (k), ice box; (l), thermometer.

to its capacity. At the same time a study was made of the progress of temperature changes in several different parts of the chambers. These temperature records were supplemented later by a more detailed study of temperature changes in a much larger number of positions in chambers filled with moistened blotters, as when the germination tests were in progress, but this time without the seeds. From these records time-temperature curves were constructed. Before considering these curves, and in order to make their significance clear, it is well to introduce a brief description of the germinating chambers.

# GERMINATING CHAMBERS

All of the germination tests were made in copper chambers surrounded, except the door, by a water-jacket space, the water in this space being the medium of temperature control. Figure 11 shows one of the chambers in median vertical section.

The interior space of each germinating chamber is approximately a 20-inch cube. A pan of water (*a*) in the bottom of this space maintains a nearly saturated atmosphere in the chamber. At intervals of 2 inches on the sides of the chamber are copper ledges (*b*) for the reception of perforated copper trays (*c*) which carry the seeds and which in the figure

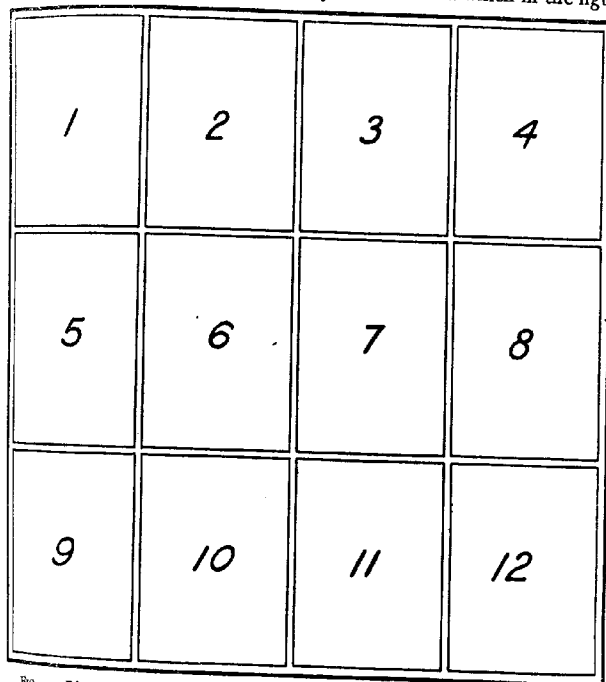


FIG. 12.—Diagram of a seed tray with folded blotters. The blotters are numbered from 1 to 12. The gas for heating the chamber passes through the thermo-regulator (*d*), which is inserted in the water jacket (*e*) and thence to the burner (*f*). The water for cooling the chamber enters the water jacket through the rubber tube (*g*) and the perforated copper sheet (*h*). The overflow passes out through the waste pipe (*i*). Whenever necessary, ice was placed in the ice box (*k*) and the water passed over it before entering the water jacket. The thermometer (*l*) indicates the temperature of the air in the extreme top of the chamber. Each seed tray except No. 8, on which bluegrass seed was tested with the use of the Jacobson apparatus, received 12 folded blotters (4 thicknesses) for the small seeds, which were tested on top of blotters, and 24 (8 thicknesses) for the larger seeds, which were tested between blotters in duplicates placed on top of each other. Figure 12 shows the arrangement of the blotters upon a seed tray.

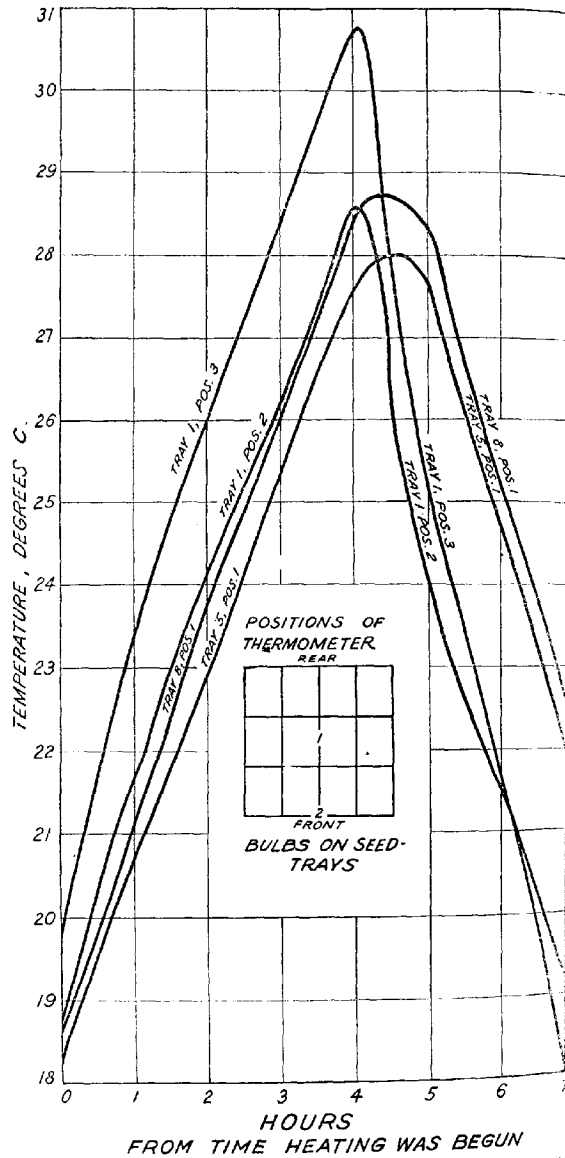


FIG. 13.—Curves showing changes of temperature on top of wet blotters in different parts of a germinating chamber which was heated by a gas flame below for four hours and then rapidly cooled by ice and cold water above for three hours.

TEMPERATURE CHANGES WITHIN THE GERMINATING CHAMBERS

As was anticipated, the temperature changes varied considerably in different parts of any given chamber. The heat imparted to the wet blotters and the seeds passed inward from the copper walls of the chamber; in cooling them the radiation was in the opposite direction. Naturally, the temperature changed more rapidly and therefore attained

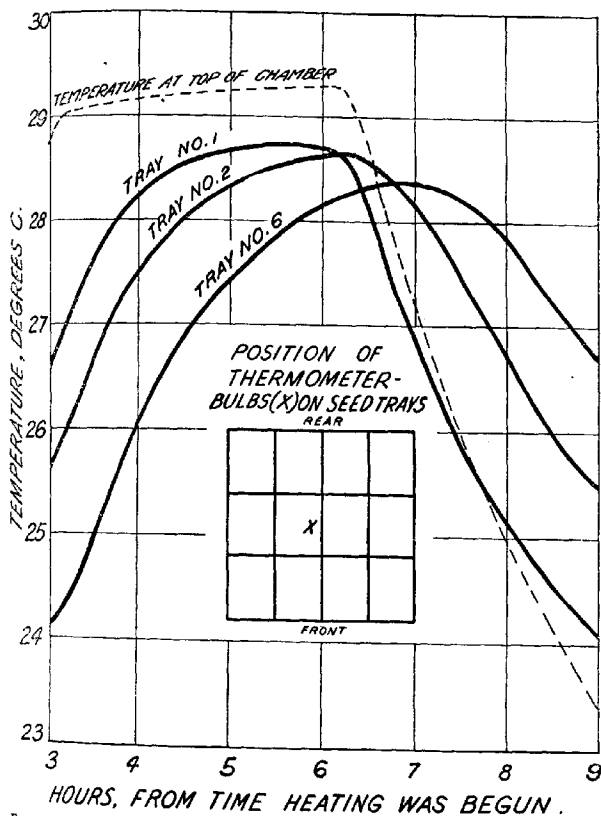


FIG. 14.—Curves showing changes of temperature within wet blotters in a germinating chamber which was heated three hours, then held at a nearly constant temperature for three hours, and finally cooled during three hours.

wider extremes near the top, bottom, and side walls of the chamber than near the center of the chamber. Furthermore, the rates of heating and cooling, especially of the seeds in the central parts of a chamber, were more rapid when only four thicknesses of blotting paper were used on each tray than when eight thicknesses were used, and were more rapid in the air between the seed trays than within the blotters themselves. These differences are illustrated in Figures 13, 14, 15, and 16.



Figure 13 shows the temperature changes as read from thermometers whose bulbs rested on top of four thicknesses of wet blotting paper in four different positions in a chamber which was heated by a gas flame below for four hours and then immediately cooled for three hours by means of ice and water above. The eight trays were completely covered with four thicknesses of folded blotters.

The curve for position 3 on tray 1 (near three walls of the water jacket) represents the most rapid and that for position 1 on tray 5 (near the center of the chamber) very nearly the least rapid temperature

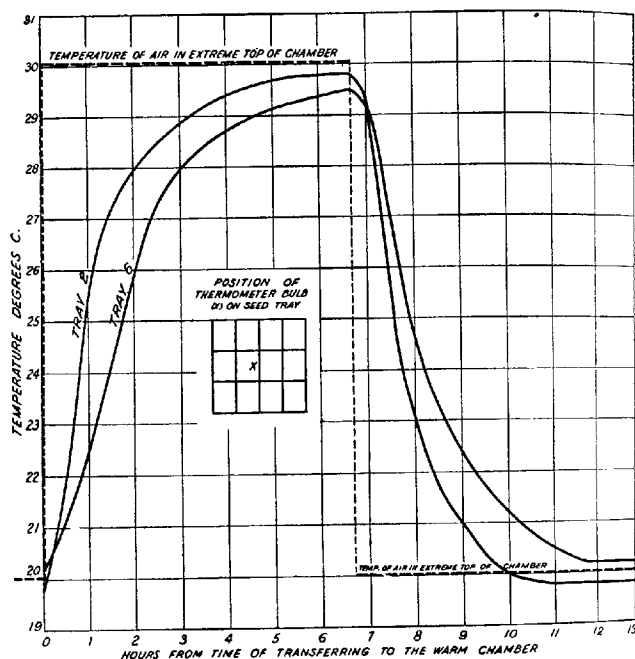


FIG. 15.—Curves showing temperature changes within wet blotters when these were transferred two germinating chambers at fixed temperatures.

changes occurring in any part of the chamber, the curve for position 2 on tray 1 (near the middle of the insulated door) represents the least rapid temperature changes for that tray; and the curve for position 1 on tray 8 (directly over the middle of the pan of water) represents a relatively slow rate of heating and cooling, combined with a period longer than in any other part of the chamber, during which the temperature was nearly constant in the neighborhood of the maximum temperature for that position.

The extremes of temperature in different parts of the chamber were 1.6° C. apart before the heating was begun, 3.2° when heating was discontinued, and 4.5° at the end of the 7-hour period. With the discontinuance of rapid cooling at the end of 7 hours, of course the temperature in different parts of the chamber became gradually equalized so that the temperature curves would converge if continued beyond the 7-hour period.

For a number of alternations the temperature of a chamber, as indicated by the thermometer in its top, was held at about its highest point for some time before cooling was begun. In these cases, as shown in

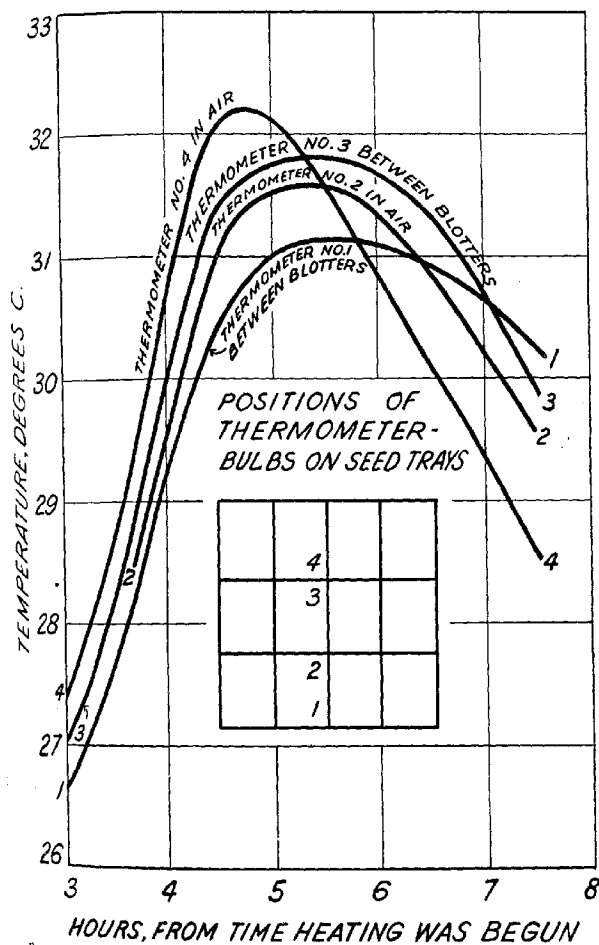


FIG. 16.—Curves showing temperature changes in four positions on top tray of a chamber which was heated and cooled. Thermometer bulbs No. 1 and 3 were within wet blotters; No. 2 and 4 were in the air about one-eighth inch above wet blotters.

Figure 14, the curves representing temperature changes within the chambers became broad and round at the top, instead of sharp and narrow as in Figure 13, and the highest temperature reached within the blotters was nearly the same in all parts of the chamber. Figure 14

shows the temperature for a number of hours within folded blotters near the middle of trays 1, 2, and 6, and also the temperature indicated by the thermometer in the top of the chamber. The effect is that of broadening the curves and displacing them to the left as viewed downward from the top tray.

Three of the eleven alternations were secured by transferring the seed trays with the seeds upon them between two germination chambers constantly maintained at fixed temperatures. In this case the temperature changes taken altogether were more nearly identical in different parts of the chambers, and approximate equilibrium in temperature between the different parts of the chambers was assumed much more quickly than in the two types of alternations previously discussed. Figure 15 shows the temperature changes within wet blotters near the middle of trays 2 and 6 in such an alternation.

Figure 16 shows much more rapid cooling of the air in the top of a chamber which is being cooled by ice and cold water above than within the blotters on the seed tray just below this layer of air. Such differences as here shown might be significant in determining whether, in a given case, a given kind of seed, for instance celery, will germinate better between blotters or on top of blotters.

#### COMPARISON OF THE DIFFERENT TEMPERATURE ALTERNATIONS

While the differences in the progress of temperature changes between different parts of a given chamber were frequently large, they were always less than the differences in the same part of different chambers which were used for different alternations. For the purpose of comparing the temperature changes in the different alternations, a position near the center of tray 6 was selected. Figure 17 shows the time-temperature curves for this position in all 11 alternations, which are numbered from 1 to 11 for convenience. The curves were drawn from actual observations for the first 15 hours and were projected for the remaining 9 hours of the day. Since the initial temperature varied in each case a few tenths of  $1^{\circ}$  C. from day to day but was always very near  $20^{\circ}$ , the curves have been slightly rectified so as to begin each at exactly  $20^{\circ}$ .

Alternations No. 1 to 3 were obtained by transferring between two chambers at fixed temperatures; No. 4 to 6, 8, and 10 by heating and then immediately cooling a single chamber; No. 7 and 9, by heating a chamber and then holding it for three hours at the highest temperature reached before beginning to cool it; No. 11 was obtained by heating a chamber and then allowing the temperature to fall naturally, without artificial cooling. The rates of heating and cooling were approximately the same from day to day in any one alternation. In all alternations after the temperature had fallen to  $20^{\circ}$  C. it was held nearly constant until heating was begun the next day.

The alternations differed widely in maximum temperature attained, mean daily temperature, and rate of cooling; but the heating was relatively rapid in all cases and the minimum temperature was the same in all cases.

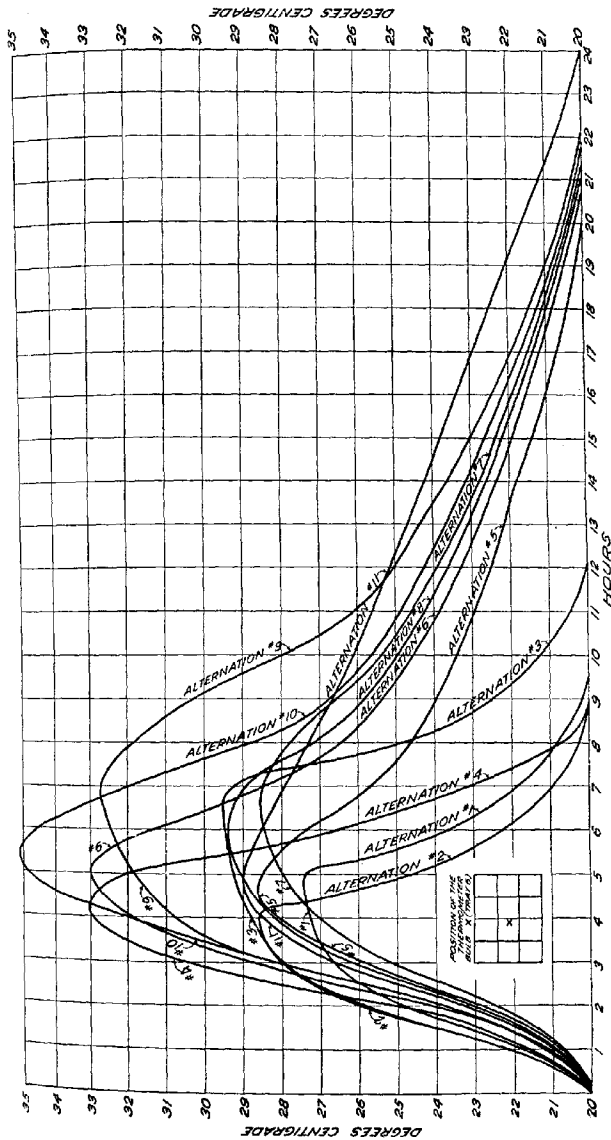


FIG. 17.—Curves showing temperature changes near the middle of tray No. 6 in 11 alternations of temperature.

## GERMINATION WITH THE DIFFERENT ALTERNATIONS

## 1. Seeds which germinated equally well with all alternations

Seeds of carrot, cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), millet (*Chaetochloa italica* (L.) Scribn.), onion (*Allium cepa* L.), parsley, redtop, sorghum (*Holcus sorghum* L.), timothy, and watermelon (*Citrullus vulgaris* Schrad.) germinated with equal completeness with all of the temperature alternations from No. 2 to No. 11. They were not tested with alternation No. 1.

Seeds of bean (*Phaseolus vulgaris* L.), beet (*Beta vulgaris* L.), upland cotton (*Gossypium hirsutum* L.), cowpea (*Vigna sinensis* (Tornei) Savi), flat pea (*Lathyrus sativus* L.), flax (*Linum usitatissimum* L.), hemp (*Cannabis sativus* L.), meadow fescue, milo (*Holcus sorghum* L.), orchard grass, pea (*Pisum sativum* L.), rice (*Oryza sativa* L.), perennial rye grass, soy bean (*Soja max* (L.) Piper), black bitter vetch (*Vicia ervilia* (L.) Willd.) were tested only with alternations Nos. 3, 5, 6, 7, and 9 but germinated with equal completeness with all of these alternations.

Some of the kinds of seed mentioned in the two preceding paragraphs have been shown in the earlier pages of this paper to germinate equally well and others almost as well at constant temperatures as with any alternation (see fig. 1, and p. 297).

## 2. Seeds which did not germinate equally well with all alternations

This class includes only three of the kinds of seed used in this part of the investigation; celery and bluegrass, which have already been shown strictly to require alternations of temperature for their best germination (see fig. 3 and 4 and text p. 299), and tomato (*Lycopersicum esculentum* Mill.). Table III shows the average percentages of germination of several lots of these three kinds of seed, together with a summary of the important features of each alternation, as shown by the time temperature curves of Figure 17. The germination period was 10 days for tomato, 26 days for Kentucky bluegrass, and 27 days for celery.

The percentage of germination of each kind of seed showed no definite correlation with the extreme or mean temperatures of the alternations or with the shape (whether narrow and abrupt or broad and rounded) of the upper part of the curves in Figure 17. There was, however, a definite relation between percentages of germination and rate of cooling, as shown by the temperature at the end of 15 hours, the number of hours of the day during which the temperature recorded was below 22.5° C., and the number of hours at 20° — changes shown by the abrupt or gradual fall of the right arm of the corresponding curves in Figure 17.

It must be admitted that the curves in Figure 17, which represent temperatures within blotters near the middle of the chambers, do not accurately represent the temperature changes occurring on top of blotters in the positions in which celery and bluegrass seeds were tested. It has already been shown, however (see fig. 13 and 17 and text, p. 320), that temperature differences in any one chamber in a given alternation were less than the characteristic differences between the different alternations. In all these tests the chambers were equally filled and each sample occupied the same position within the chamber in each test. Irregularities due to varying positions are therefore eliminated, and the conclusion is justified that an elevated temperature should not be long maintained. This agrees with the results of the reverse alternations discussed on page 305.

TABLE III.—*Germination of celery, Kentucky bluegrass, and tomato seed with 11 temperature alternations*

Alternation number.	1	2	3	4	5	6	7	8	9	10	11
Approximate maximum temperature ( $^{\circ}$ C.).....	27.5	28.5	29.5	33.0	28.5	33.0	28.5	29.5	32.5	023	59.0
Approximate mean temperature ( $^{\circ}$ C.).....	21.5	21.5	22.5	22.0	22.5	24.0	23.5	23.5	25.5	24.5	24.5
Temperature at end of 15 hours ( $^{\circ}$ C.).....	20	20	20	20	21.5	22.0	22.3	22.2	23.0	22.7	23.8
Number of hours below 22.5 $^{\circ}$ C.....	19	19	16	18	14	12	11	11	10	10	8
Number of hours at 20 $^{\circ}$ C.....	15	15	12	15	4	3	3	3	2	2	(a)
Average per cent germination (Celery:											
5 lots of good seed.....	78	76	78	74	73	72	66	67	67	43	
3 lots of poor seed.....	39	33	35	30	35	27	24	20	26	16	
Kentucky blue grass:											
7 lots.....	65	68	64	64	63	61	59	58	60	59	50
Tomato:											
4 lots.....			73		71	68	66		59		

<sup>a</sup> The temperature in this alternation sometimes did not fall to 20 $^{\circ}$  C. in the entire 24 hours.

For best germination of the three kinds of seed here considered the temperature after its initial rise to a higher degree should fall at least as low as 21.5 $^{\circ}$  C. within 15 hours from the time the heating was begun, should be below 22.5 $^{\circ}$  for at least 12 hours of the day, and should be as low as 20 $^{\circ}$  for several hours each day. These results could be attained by the method of transferring between two germinating chambers at fixed temperatures even if the seeds were kept as long as 10 hours in the warmer chamber. While such alternations were not used in this investigation the indications, both from the work on tomato seed recorded in this paper (Table III) and from Vanha's work (21), are that the longer period at the higher temperature would be injurious.

Altogether the investigation revealed an unexpectedly large degree of tolerance to a wide range of conditions in temperature alternations on the part of celery and bluegrass seed.

The method of transferring between two germinating chambers at fixed temperatures is much simpler to operate than heating and cooling a single chamber, on account of the difficulties in the way of accurate temperature control in the latter case; it involves more uniform conditions throughout an entire chamber and from day to day, and it gives at least as good results. It is therefore decidedly preferable to the other method.

### 3. Rapidity of germination

Celery and bluegrass seed germinated most rapidly with those alternations of temperature which also gave most complete germination and least rapidly with those alternations which gave least complete germination. Here, as in the earlier comparisons between alternations and constant temperatures (see p. 301), the special effect of a favorable alternation of temperatures predominates over the effect of low or high mean temperatures. This is in marked contrast to the temperature responses of constant-temperature germinators.

Parsley seed germinated more rapidly with alternation No. 5 than with a warmer alternation or with either more rapid or less rapid cooling.

All other kinds of seed germinated more rapidly with the warm than with the cool alternations. The differences were related to the rate and extent of cooling, the mean temperature, and the proportion of each day at a comparatively warm temperature, and were not related to the maximum temperature occurring in the alternation. Usually germination

was most rapid with alternation No. 9 and least rapid with alternation No. 4, although the upper temperature extreme was practically the same in the two alternations.

The germination of the seeds of the cucurbits was especially accelerated by the warm alternations. The average percentage of germination of watermelon seed in three days was 66 with alternation No. 9 and only 6 with alternation No. 4.

#### 4. Uniformity of results with favorable alternations

Some lots of seed are much more sensitive to temperature conditions than other lots of the same kind. Consequently, while the most favorable temperature conditions give with every lot approximately the maximum germination attainable, less favorable conditions (for general use) may give equally good results with some lots and very poor results with others. A really very good, but temperature-sensitive, lot of seed may thus under slightly unfavorable temperature conditions germinate more poorly than a very poor lot which is not markedly sensitive to temperature conditions. It is impossible to say just what constitutes this different temperature sensitiveness of different lots of the same kind of seed, but it frequently becomes evident. It was strikingly exemplified by the celery seed used in this investigation. This suggests the possibility that some of the kinds of seed which in this investigation germinated as well with constant as with alternating temperatures may, under some circumstances—for instance, incomplete after-ripening—germinate better with an alternation.

Figure 18 shows the germination of eight lots of celery seed with four temperature alternations Nos. 2, 3, and 4, which were very favorable alternations, and No. 11, which was unfavorable. The curve for alternation No. 5 would follow almost exactly the course of that for alternation No. 2. Celery seed No. 87602, though one of the best germinators under favorable conditions, germinated less than half as much as the poorest lot when tested with alternation No. 11.

The difference in sensitiveness of different lots to temperature conditions is even more strikingly shown in Figure 19, which shows the germination of three lots of celery seed with 10 temperature alternations.

#### COMPARISON WITH THE RESULT OF FIELD TESTS<sup>6</sup>

##### Temperature changes in the soil during the spring and early summer

Several years ago, in connection with a series of field tests of various kinds of seed, records were kept of the temperature of the soil at various depths on Potomac Flats, near Washington, D. C. The period covered by the tests was from March 26 to June 28, inclusive. Nearly every day during this period temperature observations were taken at intervals of from 15 to 30 minutes, beginning at 4.30 a. m. and continuing to about 10.30 p. m.

From March 26 to April 22 the temperature of the soil at a depth of 1 inch was always below 30° C. and on several different days did not become as warm as 20°. The period from April 22 to May 1 was somewhat warmer, but the temperature of the soil at 1 inch was never above 31°, and on one day barely reached 20°. On 18 of the 28 days in May for which there are records the daily range was from 20° or cooler to 30° or warmer. The other 10 days for which there are records were cooler. From June 1 to 7 and from June 11 to 15 the

<sup>6</sup> The data for soil temperatures and seedling production used in this comparison are from unpublished notes furnished by Mr. E. Brown, botanist in charge of the seed-testing laboratories.

weather was very warm. The temperature of the soil for the rest of June was only slightly warmer than in May.

The temperature range for 24 hours at a depth of 1 inch was nearly always more than  $10^{\circ}\text{C}$ ., averaged about  $16^{\circ}$ , and not infrequently amounted to as much as  $20^{\circ}$ . The smallest range on any day was  $6^{\circ}$  and the largest  $23.5^{\circ}$ .

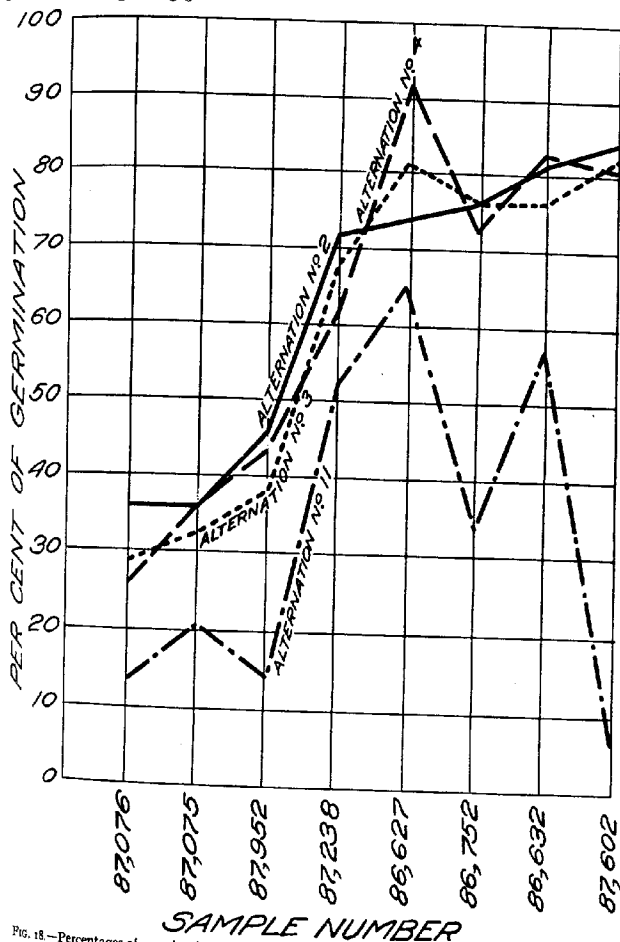


FIG. 18.—Percentages of germination of eight lots of celery seed with four alternations of temperature.

#### Seedling production in the field

Beginning with March 26, field tests were started every two or three days until early in June, seed from the same lots always being used. In each test 500 seeds of each lot were planted, and a record was kept of the number of days before the appearance of the first seedlings and of the number of seedlings appearing at intervals of a few days thereafter.



Of the seeds used in this field investigation, those of beans, beet, muskmelon, onion, parsley, peas, squash, tomato, and watermelon were included also in the chamber tests with different alternations of temperature previously discussed. It will be remembered that all of these except tomato germinated with equal completeness in all of the chamber tests but that the rapidity of germination varied according to the character of the alternation.

The bean, beet, onion, parsley, pea, and tomato seeds produced the first seedlings in the smallest number of days during the moderately warm



FIG. 19.—Percentages of germination of three lots of celery seed with 10 alternations of temperature.

weather of May or during the latter half of June, when the temperature was similar to that in May. Muskmelon, squash, and watermelon seeds produced the first seedlings in the smallest number of days during the very warm weather of the first half of June.

It should be remembered in this connection that the production of seedlings in the field involves not only the germination of the seeds but the piercing of the overlying soil by the young seedlings—a process which reflects the vigor of growth of the seedlings and may be retarded by excessively high temperatures, even when these same temperatures hasten germination as with Johnson grass seed (see p. 307). Since, however, the seedlings of the kinds of seeds here considered appeared normal and vigorous in all the alternations and also in the field, and since the chamber conditions giving most rapid germination gave also

most complete germination, the rapidity of germination in the chambers has been adopted as a basis for comparison with the promptness of seedling production in the field. The total percentage of seedling production is not considered here, because it is dependent upon other factors besides temperature conditions and does not allow of reliable comparisons in this connection.

#### COMPARISON OF SOIL TEMPERATURES WITH TEMPERATURES IN GERMINATING CHAMBERS

Figure 20 shows the time-temperature curves of three of the alternations which are represented in Figure 17, in comparison with those for the soil at the depth of 1 inch for four of the days during which the field tests were in progress.

The curves for April 16 and April 29 show the widest and the narrowest daily range in soil temperature occurring during the investigation. Both of these days came within a cool period of very slow seedling production. The curves for May 18 and June 27 fairly represent the soil temperature during the 2 periods—the larger part of the month of May and the latter part of June—during which the majority of the kinds of seed produced seedlings most promptly.

The soil temperature curves for May 18 and June 27 are similar in shape and range to those for chamber temperatures yet show some characteristic differences: (1) The lower temperature is well below  $0^{\circ}\text{C}$ . and the range is therefore greater; (2) the rate of cooling is less rapid than in case of the alternations which were secured by transfer between two chambers (No. 3) and much more rapid after about 5 p. m. than in the case of the alternations which were secured by heating and cooling a single chamber (Nos. 6 and 9), except in the case of No. 4 (fig. 17). Both of these soil temperature curves conform to the requirements set or temperature alternations in chamber tests of Kentucky bluegrass, clover, and tomato seed (see p. 322)—that is, the temperature falls below  $11.5^{\circ}$  in less than 15 hours after it first rises above  $20^{\circ}$  and is below  $12.5^{\circ}$  for more than 12 hours and as low as  $20^{\circ}$  for several hours of the day. Besides, the approximate mean temperatures for the day ( $23^{\circ}$  May 18 and  $23.5^{\circ}$  June 27) are just about the same as for some of the more successful alternations, as shown earlier in this paper, and are less than for the alternations Nos. 9, 10, and 11, which gave lower total germination, and in which the temperature remained relatively high during a large part of the day.

#### COMPARISON OF TEMPERATURE CONDITIONS GIVING MOST RAPID GERMINATION IN THE CHAMBERS WITH THOSE GIVING EARLIEST SEEDLINGS IN THE FIELD

The smallest number of days required for the production of seedlings in the field was 4 days for bean and muskmelon seeds, 5 days for beet, pea, squash, and tomato seeds, 6 days for watermelon seeds, 8 days for onion seeds, and 12 days for parsley seeds. The average daily range in the temperature of the soil at a depth of 1 inch during this period of most rapid seedling production was determined by adding separately the maximum temperatures and the minimum temperatures for the separate days and dividing the sums by the number of days. Table IV compares these temperature ranges with those occurring in the chambers in case of the temperature alternations giving most rapid germination and shows also in each case the approximate number of hours of each day during which the temperature remained below  $22.5^{\circ}\text{C}$ .

The average daily range of temperature for most rapid seedling production in the soil was in every case greater than for most rapid

germination in the chamber tests. With all except the cucurbits (musk-melon, squash, and watermelon) the average daily minimum was from  $2^{\circ}$  to  $6^{\circ}$  C. cooler in the soil than in the chambers. The average daily maximum was approximately the same in the soil as in the chamber in case of bean, beet, and pea seeds;  $3^{\circ}$  cooler in case of onion seeds; and  $3.5^{\circ}$  warmer in case of parsley and tomato seeds.

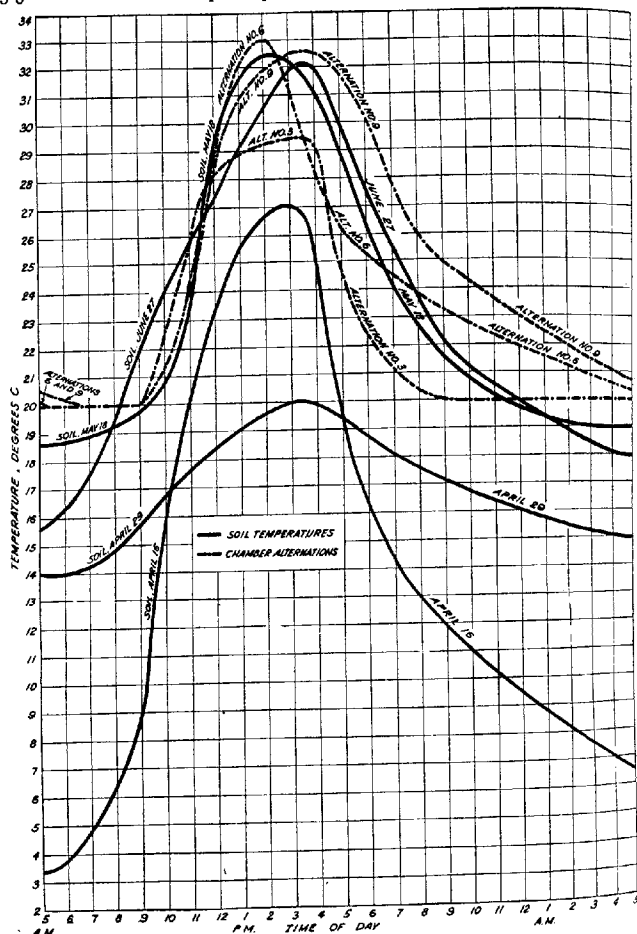


Fig. 30.—Curves showing soil temperatures for four days and chamber temperature with three alternations

With the seeds of the cucurbits the average daily minimum temperature of the soil when seedlings appeared most promptly was the same as or warmer than that occurring in the chamber tests, and the average daily maximum was  $7^{\circ}$  or  $8^{\circ}$  C. warmer than the maximum in the chamber test giving most rapid germination, although the mean temperature of this alternation was warmer than that of any other.

TABLE IV.—Comparison of temperature conditions giving most rapid germination in the chambers with those giving earliest seedling production in the field

Kind of seed.	Place of test.	Daily range of temperatures giving most rapid germination or earliest seedling production. ° C.	Number of hours below 22½° C.	Number of temperature alterations.*
Bean.....	Chamber.....	20 to 33	10	9
	Soil.....	18 to 32	12	.....
Beet.....	Chamber.....	20 to 33	10	9
	Soil.....	18 to 32	11	.....
Muskmelon.....	Chamber.....	20 to 33	10	9
	Soil.....	20 to 40	9	.....
Onion.....	Chamber.....	20 to 33	10	9
	Soil.....	14 to 30	16	.....
Parsley.....	Chamber.....	20 to 28.5	14	5
	Soil.....	17 to 32	12	.....
Pea.....	Chamber.....	20 to 33	10	9
	Soil.....	16 to 33	14	.....
Squash.....	Chamber.....	20 to 33	10	9
	Soil.....	23 to 41	3	.....
Tomato.....	Chamber.....	20 to 28.5	11	7
	Soil.....	18 to 32	11	.....
Watermelon.....	Chamber.....	20 to 33	10	9
	Soil.....	20 to 40	8	.....

\* See figure 17.

## CONCLUSIONS SUGGESTED BY THE FOREGOING COMPARISONS

Altogether Table IV and Figure 20 show a close correspondence between chamber temperatures and soil temperatures which gave most prompt germination of the seeds used. Undoubtedly a similar correspondence exists between the temperature conditions best fitted for artificial germination tests of Bermuda grass and Johnson grass seed (fig. 5 and 6 and text p. 304-305) and the soil temperatures during the period in which they germinate in nature in the warm climate of the places to which they are native.

The results of the comparison suggest:

1. For general use in conducting germination tests an alternation of temperatures covering a somewhat wider temperature range than the customary alternation between 20° and 30° C., for instance an alternation between 20° and 32° C. However, if the alternation is secured by the method of transfer between two germinating chambers at fixed temperatures, probably better results would be obtained with many kinds of seed by the use of a temperature range less than that characteristic of conditions in the soil, where the temperature changes are less rapid and the period during which the temperature is near either extreme is shorter. In view of these considerations and the additional fact that the warmer alternations Nos. 9 and 10 though giving more rapid germination in many cases, were less favorable in other cases than the cooler alternations, an alternation similar to No. 3 seems to meet the theoretical requirements of approximating with sufficient accuracy what may be termed optimal field conditions.

2. For use in germination tests of onion seed an alternation of temperatures covering a cooler range than that of any of the alternations with which it was tested in this investigation.

3. For the germination of seeds of the cucurbits an alternation of temperatures covering a warmer range than any of those investigated.

Whether or not any advantage would be derived from any of these suggested changes in method remains for further experiment to show.

## SUMMARY

(1) Seeds of carrot, parsley, timothy, awnless brome grass, perennial and Italian rye grasses, meadow fescue, and several kinds of flower seeds germinate practically as well at favorable constant temperatures as with an alternation of temperatures.

(2) Seeds of redtop and parsnip and sometimes seeds of petunia germinate somewhat better, and seeds of celery, orchard grass, Kentucky bluegrass, Bermuda grass, and Johnson grass germinate much better with favorable alternations of temperatures than at constant temperatures. The exact alternation giving best results depends upon the kind of seed and to some extent also upon its physiological condition.

(3) Different lots of the same kind of seed sometimes vary widely in temperature sensitiveness. It may therefore be that some kinds which are usually constant-temperature germinators may, under certain conditions, germinate better with an alternation of temperatures. Incomplete after-ripening might have this effect.

(4) Several hypotheses have been suggested which may help to explain the effect of alternating temperatures upon germination, but none of them can be considered adequate without more definite evidence than is now available.

(5) The favorable effect of an alternation of temperatures upon the germination of certain kinds of seed can not, with our present knowledge, be referred to the specific effect of the extreme temperatures of the alternation or of the mean temperature of the alternation but are the result of the changes in temperature. In the case of Kentucky bluegrass and Johnson grass seed, at least in some cases, the effect of the mean temperature of the alternation supplements the effect of the alternation as such.

(6) On account of greater ease, simplicity, and uniformity of temperature control, coupled with equally good germination results, the method of securing temperature alternations by transfer between two germinating chambers at fixed temperatures is preferable to the method of heating and cooling a single chamber.

(7) In the use of temperature alternations the upper temperature should be maintained only a small part of the day, never more than eight hours and usually not more than six hours, and the change to the lower temperature should then be fairly rapid. These results are easily attainable by the transfer method.

(8) An alternation between 20° C. for 16 to 18 hours and 30° for 6 to 8 hours each day gives good results in the germination of parsnip, celery, redtop, and orchard-grass seed and seems to be an optimum temperature condition for germination of Kentucky bluegrass seed.

(9) A large percentage of at least some lots of celery seed will germinate at nearly constant low temperatures (15° to 20° C.), but germination is then so slow as to make the use of a warmer alternation preferable.

(10) A similar alternation between 20° and 35° C. gives good results with Bermuda grass seed; an alternation in a still warmer temperature range might possibly be still better.

(11) For the germination of Johnson grass seed the very warm alternation 30° C. for from 18 to 22 hours and 45° for from 2 to 6 hours each day is best.

(12) Those lots of Johnson grass seed which germinate most readily under favorable temperature conditions are less sensitive to temperature—that is, they germinate well under a wider range of temperature con-

ditions—than those lots which germinate less readily even under the most favorable conditions.

(13) A large percentage of some lots of Johnson grass seed will go through at least the first visible stages of germination and some of the seeds will develop apparently healthy seedlings at warm constant temperatures (35° to 40° C.), but a part of the seedlings are abnormal and capable of only slight development at these temperatures while the percentages of germination remain below those for favorable alternations of temperature.

(14) With alternations between 25° and 40° C. or between 30° and 45° the length of time from two hours to eight hours during which the seeds are kept in the warmer chamber has little effect on the percentage of germination of fully after-ripened Johnson grass seed but affects the rate of germination somewhat more.

(15) Removal of the scales from Johnson grass caryopses greatly increases their germination under relatively unfavorable temperature conditions, somewhat increases their germination with the most favorable temperature alternations, and considerably accelerates their germination in both cases.

(16) The temperature changes to which the seeds are subjected in any given alternation vary with their position within the germinating chamber and with the method of testing (whether between or on top of wet blotters) and are in no case exactly the same as indicated by a thermometer inserted into the air in the top of the chamber.

(17) The temperature changes giving best germination results with a large number of kinds of seed correspond rather closely with soil temperatures in the field under conditions which induce the most prompt and vigorous production of seedlings. A similar correspondence undoubtedly exists between the temperature conditions, giving best results with Bermuda grass and Johnson grass seed on the one hand and soil temperatures in the warmer localities to which these grasses are native on the other hand.

(18) The results of field tests suggest the use of an alternation between about 18° and about 32° C. for the germination of a large number of kinds of seed; an alternation covering a lower temperature range than 20° to 30° with onion seed; and an alternation covering a considerably higher temperature range with seeds of the cucurbits. Without experimental evidence, however, it is impossible to say whether or not any of these alternations in the cases indicated would have any advantage over an alternation between 20° and 30°.

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## WATER CONTENT OF BARLEY KERNELS DURING GROWTH AND MATURATION<sup>1</sup>

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### INTRODUCTION

In studies in kernel development during the last few years close records have been kept of a number of variables. Several of these studies already have been published.<sup>2</sup> Of all of the variables observed the behavior of water has been the most consistent. As shown in 1920,<sup>3</sup> the percentage of water by days from the date of flowering was practically identical in two different seasons at Aberdeen, Idaho, and showed almost no fluctuation. When plotted, the percentage was essentially a straight line starting at about 82 per cent and ending at about 48 per cent. Below 48 per cent the curves were not coincident. The uniformity of these results indicates a closely vital relation of water content with growth and maturation. The results previously reported have been based on the averages of a varying number of spikes per day. The wet weights of the kernels were secured individually, but the dry weights were obtained on the aggregate kernels of each spike. In the studies here reported the dry weight of each individual kernel was obtained, so that individual dry weights and percentages of water of individual kernels are available for the first time. For certain purposes these data have been totaled as before.

### MATERIAL AVAILABLE

Several varieties were studied in detail. The earlier attempts were with hulled varieties. These were found to be unfit because it becomes impossible to remove the lemma and palea once mechanical drying has begun. In these varieties the sampling became impossible later than this period and, therefore, the final incidents of maturation were lacking. The results with naked varieties only are reported in this paper. The growth tables of three growing periods were available for interpretation. These include two seasons, 1919 and 1920, with the Baku variety, and one season, 1920, with the Jet. The Baku is a white, naked 2-rowed

<sup>1</sup> Accepted for publication May 20, 1922. These studies were made on the Aberdeen Substation, Aberdeen, Idaho, in connection with cereal experiments conducted cooperatively by the Idaho Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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variety originating near the Caspian Sea. The Jet is a 2-rowed naked variety from Abyssinia which develops a very black pigment in the pericarp at maturity. As the occurrence of this pigment is thought to be of significance in the later stages of maturation, the Jet variety is considered the basic one in the discussion which follows. The growth

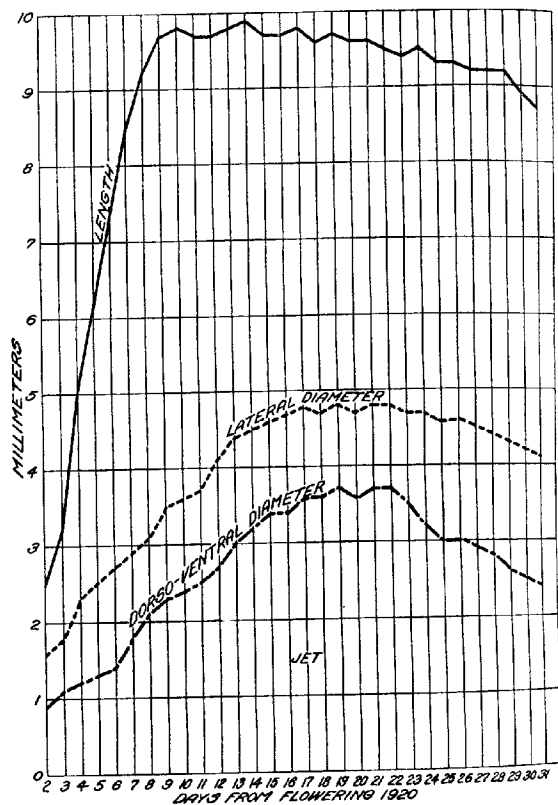


FIG. 1.—Average length, lateral diameter, and dorsoventral diameter of kernels of Jet barley at Aberdeen, Idaho, in 1920.

and maturation of the Baku variety vary somewhat from those of the Jet. These differences are apparent in the tables and figures and are pointed out in the discussion. In the figures which give data from the Baku variety for both 1919 and 1920, the time from flowering is for the 1920 data. The Baku variety in 1919 is advanced three days to avoid confusion of the lines. The shorter growing season of the Baku variety in 1919 was doubtless the direct result of drought, as the water for irrigation was insufficient in that year.

## SUMMARY OF PREVIOUS INVESTIGATIONS

In papers previously published it has been pointed out that various phenomena occur with remarkable uniformity during the development of the barley kernel. The length, lateral diameter, and dorso-ventral diameter increase, attain their maximum, and decrease toward maturity

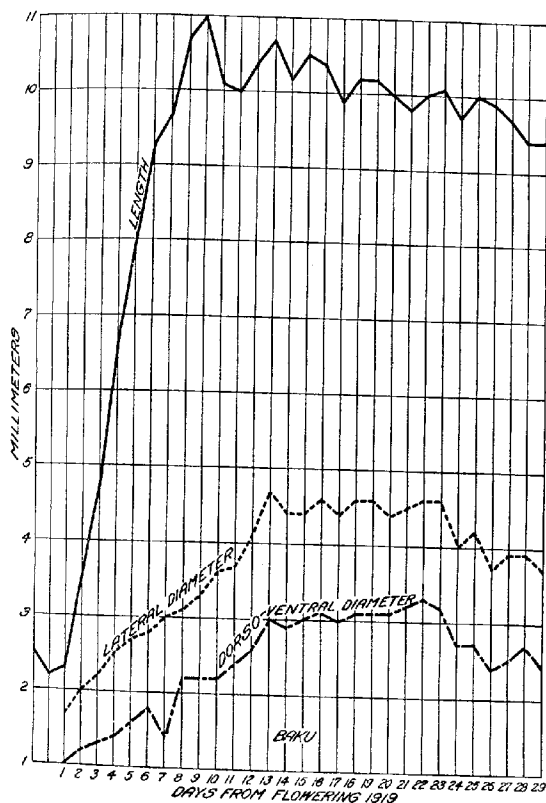


FIG. 2.—Average length, lateral diameter, and dorso-ventral diameter of kernels of Baku barley at Aberdeen, Idaho, in 1919.

in very definite order. The wet weight increases rapidly and then decreases, as does the total water. The percentage of water decreases very uniformly from flowering to maturity. In the previous studies, where the unit has been the spike, maturation has occurred when about 42 per cent of water is present in the spike. This was necessarily an average figure which included kernels well past maturity and kernels which had not yet ceased to function. As may be seen in figure 1, the length of the Jet variety, in 1920, was reached by 9 days after flowering,

after which it remained almost constant until maturity. The lateral diameter had almost completed its growth by the fourteenth day and remained stabilized until the twenty-second day. The dorsoventral diameter increased until the eighteenth day, although the rapid increase was completed by the fifteenth day. The diameter was maintained until

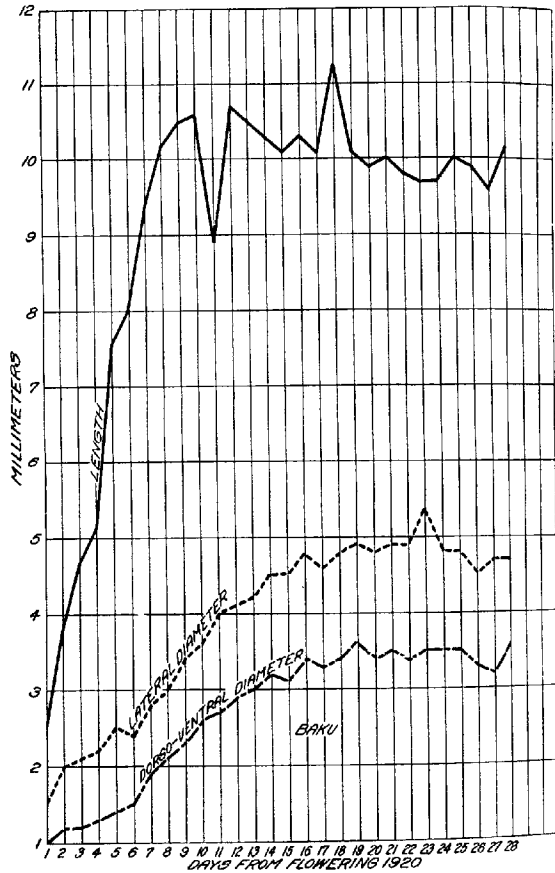


FIG. 3.—Average length, lateral diameter, and dorsoventral diameter of kernels of Baku barley at Aberdeen, Idaho, in 1920.

the twenty-second day. The wet weight increased rapidly for 17 days, with very slight continued increase until the twenty-second day. The total water increased rapidly for 16 days and showed no active loss until the twenty-second day. The dry weight increased rapidly until the twenty-second day and very gradually from then until the thirtieth. The percentage of water decreased gradually until the twenty-second day, rapidly from then until the twenty-seventh, and abruptly from the

twenty-seventh to the thirty-first, at which time the kernels had reached the moisture content normal to stored grain. As may be seen in Figures 2 and 3, the Baku variety reached these various stages at a slightly later date. In other words, the growing period of Baku was 2 or 3 days longer than that of Jet.

#### TOTAL WATER CONTENT OF JET AND BAKU VARIETIES

The average water content of the kernel of Jet, as in other varieties of barley, shows, when plotted, a very different curve from that of

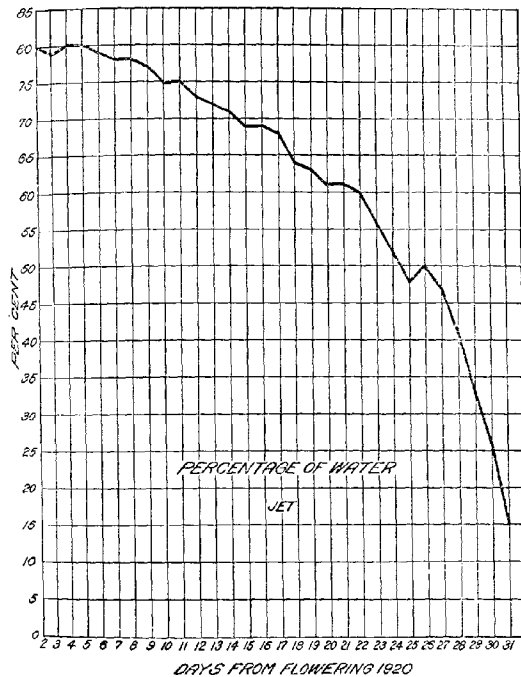


FIG. 4.—Average percentage of water in kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

percentage of water. While not as perfect as in the Hannchen variety reported in 1920, the curve of percentage of water in Jet (fig. 4) descends in an almost straight line during the period of active growth. On the other hand, the curve of total water (fig. 5) exhibits three distinct phases. For a few days after fertilization there is a rapid increase in the water content of the kernel. There then follows a period of a few days in which the water content is almost stationary. Following this there is a uniform decline until maturity is reached and a more rapid decline to the point approximating the water content of the stored grain of the region. Stated in another way, for a period of about 12 days there is an increase

of water content simultaneous with a deposit of starch, the latter beginning the fourth day after flowering.

During the second stage there is little change in water content, but there is a heavy increase in starch content—that is, there is a deposit of starch which is not displacing water. The volume of the kernel

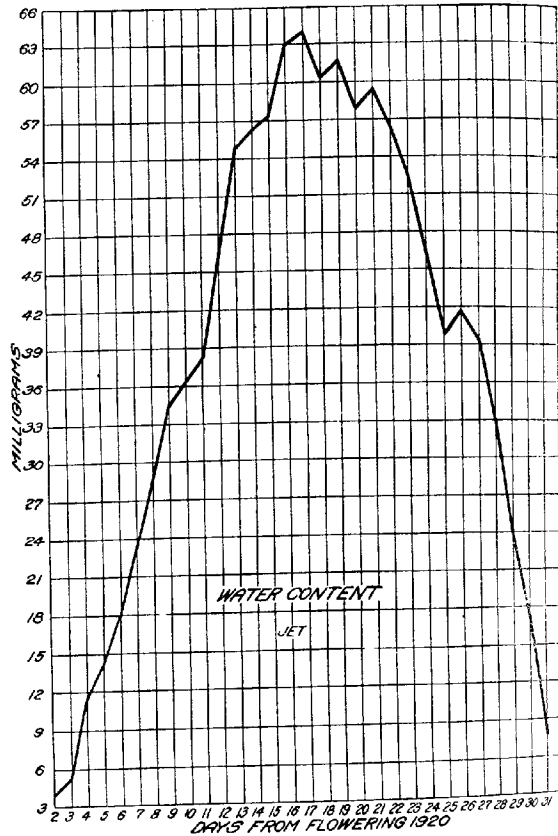


FIG. 5.—Average total water content of kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

continues to increase during this period. The increase in volume must come either from the increase in number of cells in the endosperm or from the increase in size of cells already formed. Most of the expansion is probably of the latter nature. The increase in volume is quite evident in figures 1, 2, and 3, which show the length, lateral diameter, and dorso-ventral diameter by days for both varieties.

The third stage, beginning about the twenty-second day in the Jet variety, shows a decided loss of water. While the rate of starch deposit

diminished as shown by the dry weight, material continued to be added for several days. The water content drops more abruptly and somewhat in advance of the wet weight, due, of course, to the continued activity of starch deposit.

The actual modifications which occur within the kernel during these changes have not been traced definitely. As the bulk of the kernel is endosperm tissue, the observable variations are doubtless dependent on a phase of endosperm development. The endosperm starts to develop about the center of the nucellus. As growth proceeds, new cells are added by repeated division of the endosperm cells first laid down. At some stage of growth the addition of these new cells is either diminished rapidly in the number added per day or, more probably, is interrupted

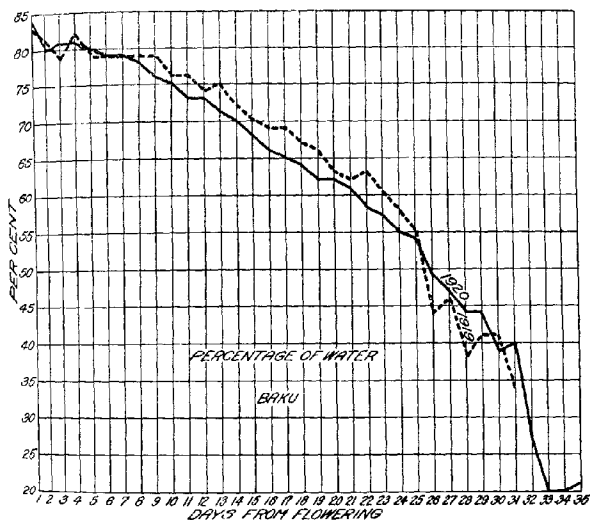


FIG. 6.—Average percentage of water in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920.

entirely except in the region immediately adjacent to the furrow. Later, two other developments are possible. As previously pointed out, there is a secondary stage of starch formation where small starch kernels appear among the large starch kernels of the first starch deposit. It is also likely that some of the cells of the starch endosperm are abandoned as places of active starch deposit. This certainly occurs in occasional cells of the periphery on the dorsal surface most remote from the furrow. Cells in the latter region can be observed which by their cell walls indicate an age equal to that of adjacent cells but which have very few starch grains in their interior. Such cells are, in a way, abnormal, and if there is any abandonment that occurs as a phase of maturation, it is of cells of normal starch content. In any case it is likely that the stages of endosperm development have a direct relation to the changes exhibited

in the wet weight, dry weight, and total water content of kernels. Judged by these indicators all varieties follow about the same course. Though Baku requires a day or two longer in maturing, its water content, as shown in figures 6 and 7, and its wet weight and dry weights, as shown in figures 8 to 11, are quite comparable to those of Jet.

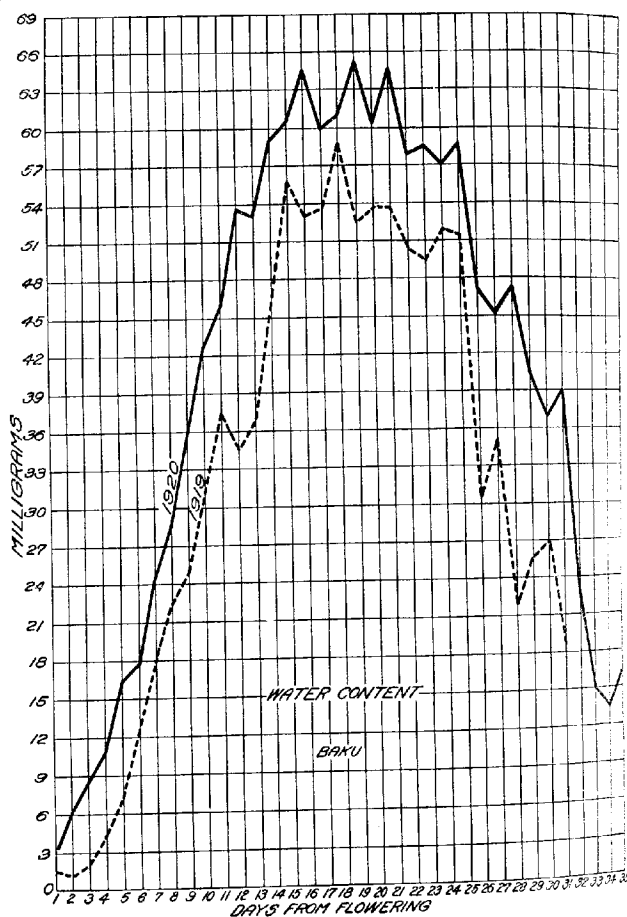


FIG. 7.—Average total water content in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920. 178 JA

#### WATER CONTENT AND DRY WEIGHT OF INDIVIDUAL KERNELS

In Table I are given the complete records of the individual kernels of these two varieties for the period under observation. While the number of observations is so great as to make a direct digest of these data difficult, a number of facts are evident. In the table the kernels are

numbered on each spike. One side of the spike only was studied, as the error of sampling was less when a larger number of spikes were included, the daily number of observations being necessarily limited by the time required to obtain the data. The kernels are numbered from the base to the tip of the spike, No. 1 being the basal kernel, No. 2 the next above, and Nos. 6 to 11 the tip kernel, depending on the length of the spike. It

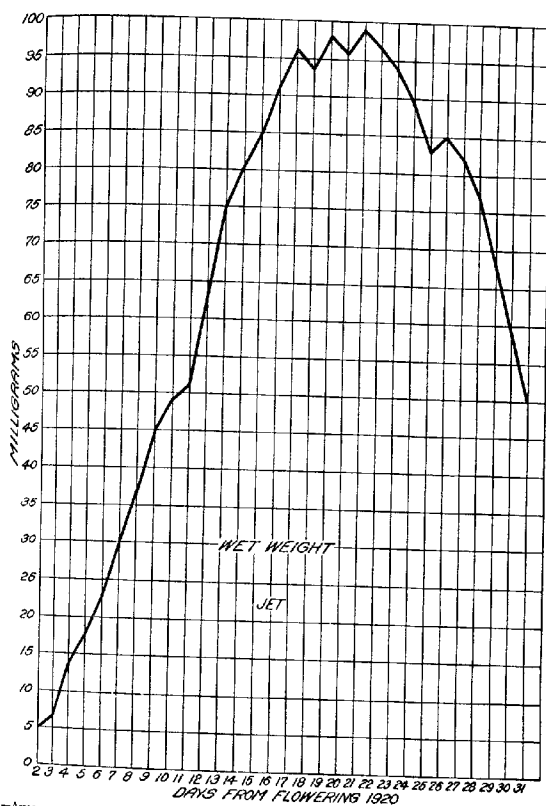


FIG. 8.—Average wet weight of kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

can be readily seen that the tip kernels never attained the size of those about the center of the spike. This fact has been frequently pointed out. As indicated by the percentage of water, it is quite noticeable that the kernels at the tip of the spike ripened much earlier than those near the base. The basal kernel itself is more nearly related to the tip kernels in that its average size is less than those above it and its maturation earlier. For the purpose of interpreting the table, any kernel under 42 per cent may be considered mature.



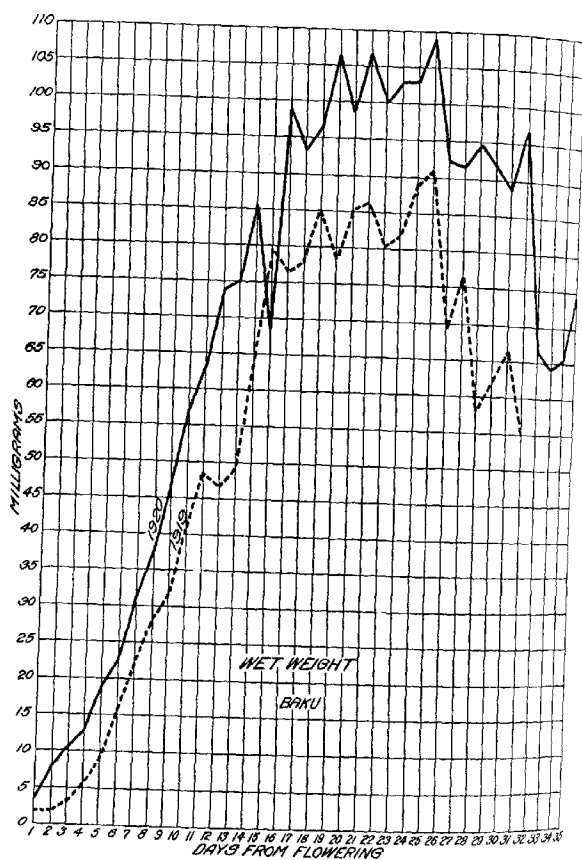
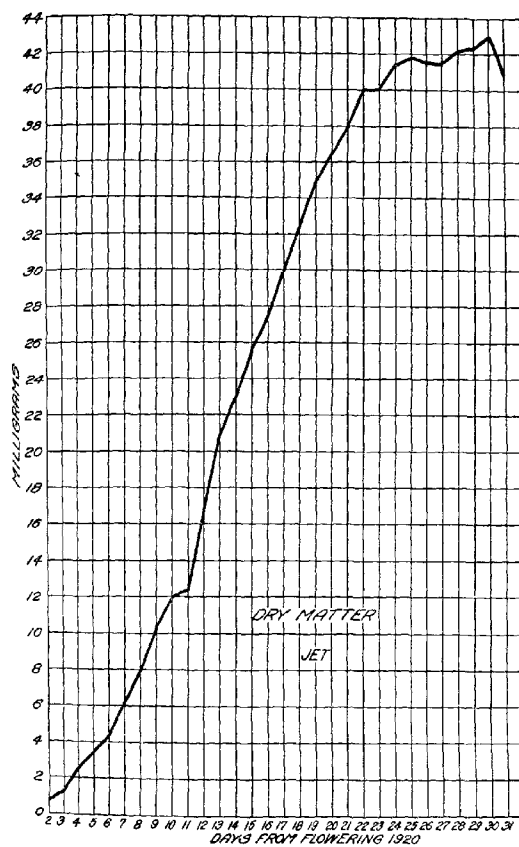


FIG. 9.—Average wet weight of kernels of Baku barley from flowering to maturity at Aberdeen, Idaho in 1919 and 1920.



6. to.—Average dry matter in kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

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Section 3 of the table differs from sections 1 and 2 in that the spikes of a single day are of different ages. It was thought that daily weather variations might be overcome if samples of varying ages were taken on each day. Spikes marked A before August 4 flowered on July 6, all those marked B, and those marked A after August 4, on July 7, and those marked C, D, and E, on July 9. In the figures showing the distribution by days from flowering and in the various averages, spikes

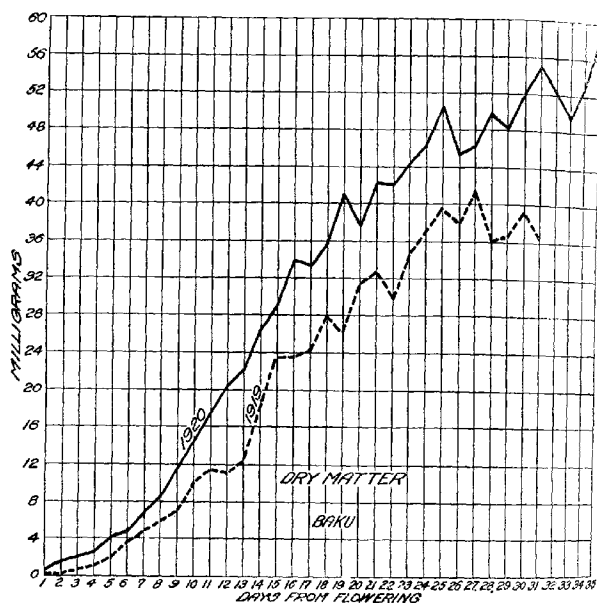


FIG. 11.—Average dry matter in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920.

of the same age are grouped together. In computing data for figures, small abnormal tip and basal kernels were omitted where it was thought the abnormality was misleading. The data in the table have been plotted as frequencies in figures 12 to 15.

The frequencies of the dry weights (fig. 12) are not much more easily interpreted than the table itself. This is due to the fact that the final weights of the kernels on the spikes vary over a considerable range. The tip kernels begin to ripen about the twenty-first to twenty-third day and are found distributed to the left of the general mass of kernels from weights of 275 mgm. to 375 mgm.

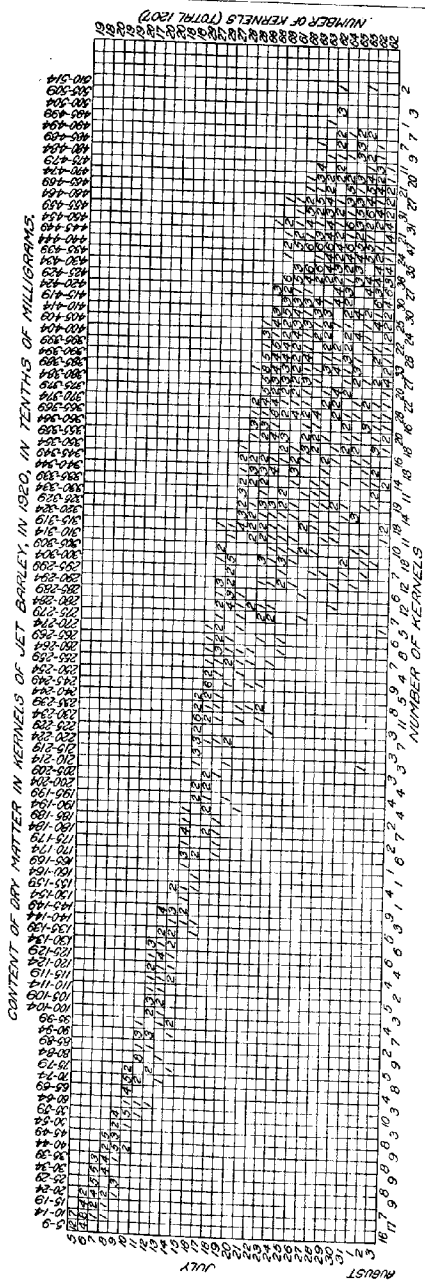


FIG. 12.—Distribution of kernels of jet barley according to the dry-matter content by day from flowering to maturity at Aberdeen, Idaho, in 1922.

TABLE 1.—Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho

SECTION 1: JET, 1920

[illegible]

[illegible]

TABLE 1.—Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho—Continued

SECTION 1: JET 1930—continued.

Date.	Spike.	Dry weight of kernels (mgm.).										Water in kernels (per cent).											
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
July 29.	A	38.1	42.4	43.3	41.8	41.9	36.9	37.3	35.2	29.2	.....	.....	51.9	50.0	50.1	46.3	47.4	41.6	48.6	47.8	48.3	.....	.....
	B	49.2	51.4	50.2	49.4	47.8	44.8	37.9	30.9	.....	.....	.....	53.1	52.6	52.3	51.6	52.7	50.4	49.7	49.7	.....	.....	
	C	49.2	50.4	50.1	45.6	44.5	41.5	40.6	38.9	31.7	.....	.....	52.9	52.6	53.0	53.1	50.5	52.7	51.4	51.5	50.8	.....	.....
	D	44.4	45.4	44.2	43.0	42.1	42.7	39.9	37.6	32.8	.....	.....	50.2	54.4	51.7	45.7	41.0	48.4	46.8	51.9	51.1	.....	.....
	E	47.8	48.3	47.6	43.2	44.0	44.4	43.5	38.0	38.3	33.9	.....	50.1	49.7	48.8	46.7	45.5	45.8	48.9	48.3	49.3	50.1	.....
30.	A	31.9	41.8	42.0	42.2	43.0	34.0	39.1	35.1	32.7	.....	.....	52.0	51.0	50.6	47.3	43.6	42.6	39.1	38.4	40.8	.....	.....
	B	45.5	46.9	46.1	45.2	46.9	40.9	38.1	36.9	39.8	30.4	.....	48.5	46.1	46.1	41.7	40.8	40.4	37.2	41.5	40.8	.....	.....
	C	39.7	46.8	46.7	43.7	45.1	44.9	44.3	43.5	39.6	33.6	.....	54.3	54.2	53.4	52.9	52.9	49.9	46.9	45.2	44.0	.....	.....
	D	37.6	43.7	43.4	42.5	42.2	40.9	39.6	38.6	35.0	30.2	.....	55.3	49.9	48.4	47.8	44.9	44.2	40.7	44.2	45.6	45.0	.....
	E	41.3	46.7	46.0	46.0	45.2	43.1	38.6	35.8	.....	.....	.....	53.8	53.4	53.9	50.6	48.1	46.9	46.6	47.1	46.0	.....	.....
31.	A	36.8	46.2	46.0	46.0	45.2	44.1	41.3	39.0	35.1	.....	.....	52.3	54.6	54.5	49.9	49.4	43.2	42.5	42.1	43.0	.....	.....
	B	30.6	48.8	49.4	48.1	48.2	45.2	39.6	35.6	32.2	.....	.....	50.4	38.3	46.2	48.1	46.0	43.3	43.1	43.2	43.6	39.5	.....
	C	44.5	48.5	48.8	46.6	48.4	46.6	43.4	40.9	37.0	.....	.....	43.8	41.4	40.5	37.8	35.9	46.4	45.0	45.1	36.0	20.4	.....
	D	38.4	47.0	47.0	46.6	45.4	44.4	40.6	38.9	38.5	35.0	29.3	.....	39.4	39.1	39.3	40.7	42.0	39.6	37.7	38.6	21.6	.....
	E	43.8	44.0	45.7	44.3	43.3	40.0	38.9	38.5	35.0	29.3	.....	39.4	39.1	39.3	40.7	42.0	39.6	37.7	38.6	21.6	.....	.....
Aug. 1.	A	40.4	51.0	49.3	46.0	45.5	42.9	42.9	42.3	38.1	36.1	39.9	47.4	41.8	42.8	43.5	39.1	38.3	37.0	39.4	37.9	36.8	38.8
	B	37.4	45.8	46.4	46.9	44.7	42.6	42.2	42.4	40.0	36.0	.....	37.5	45.6	46.0	39.9	31.0	26.8	18.7	15.4	18.4	21.2	
	C	31.3	42.8	45.6	45.2	43.9	40.8	45.1	42.5	38.3	33.1	.....	20.0	26.8	31.9	28.6	31.0	26.8	18.7	15.4	18.4	21.2	
	D	40.8	48.1	48.4	46.4	46.9	43.6	42.2	42.4	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	E	44.4	47.2	46.5	44.4	44.4	43.0	43.6	43.6	42.4	34.4	.....	39.4	37.1	37.5	38.8	35.5	33.6	31.4	35.1	38.9	.....	
3.	A	29.5	37.5	41.9	44.4	43.0	41.2	40.7	36.9	32.5	.....	.....	37.6	34.2	34.8	35.8	32.5	31.6	28.9	.....	.....	.....	
	B	40.8	48.1	48.4	46.4	46.9	43.6	42.2	42.4	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	C	35.3	47.9	45.8	48.8	47.1	46.5	42.1	38.2	34.2	.....	.....	32.3	39.6	37.8	30.8	26.0	24.7	20.5	18.7	15.4	21.2	
	D	43.3	46.1	45.7	45.0	44.1	39.3	38.0	35.0	31.0	.....	.....	18.5	24.0	22.3	20.8	16.0	13.5	13.8	13.4	13.9	.....	
	E	43.0	47.6	45.9	45.0	44.4	40.0	39.6	36.9	32.5	.....	.....	18.5	24.0	22.3	20.8	16.0	13.5	13.8	13.4	13.9	.....	.....
3.	A	41.7	44.7	46.3	45.4	45.9	43.5	43.5	43.5	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	B	30.7	45.5	45.8	45.8	45.8	43.6	42.2	42.4	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	C	41.7	44.7	46.3	45.4	45.9	43.5	43.5	43.5	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	D	30.7	45.5	45.8	45.8	45.8	43.6	42.2	42.4	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	
	E	41.7	44.7	46.3	45.4	45.9	43.5	43.5	43.5	40.0	36.0	.....	14.8	36.6	33.1	33.1	31.0	26.8	18.7	15.4	18.4	21.2	

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho—Continued*

SECTION 2: BAKU, 1919

	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)	(L)	(M)	(N)	(O)	(P)	(Q)	(R)	(S)	(T)	(U)	(V)	(W)	(X)	(Y)	(Z)
1.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
2.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
3.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
4.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
5.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
6.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
7.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
8.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
9.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
10.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
11.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
12.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
13.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
14.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
15.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
16.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
17.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
18.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
19.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z







TABLE 1.—Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho—Continued

SECTION 3: BAKU, 1920.

Date.	Spike.			Dry weight of kernels (mgm.).											Water in kernels (per cent).										
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11			
July 10.	A	0	...	1.7	2.0	2.3	2.3	2.1	2.0	1.7	1.0	80.8	...	80.2	79.2	79.3	79.6	80.7	80.6	79.6	78.7	77.8	77.8		
	B	5	0.7	1.4	1.6	1.9	2.1	1.8	1.9	1.7	1.1	80.8	84.1	80.2	81.4	81.0	80.9	80.3	80.6	80.6	81.7	82.8			
11	A	1	...	1.6	1.9	2.1	2.1	1.8	1.8	1.7	1.1	77.7	...	80.2	80.2	80.2	80.2	80.2	80.2	80.2	80.2	80.2			
	B	10	2.1	2.6	2.7	3.1	3.3	3.3	3.0	2.0	5	81.4	81.2	81.3	81.3	81.3	81.3	81.3	81.3	81.3	81.3	81.3			
22	A	7	0	1.6	1.7	2.1	2.7	1.7	1.7	1.6	1.3	80.9	...	80.6	79.7	79.3	78.7	79.3	80.0	79.7	78.9	78.9			
	B	24	3.8	4.0	5.7	5.5	5.3	5.1	4.1	3.2	...	80.9	79.3	79.3	79.3	79.3	79.3	79.3	79.3	79.3	79.3				
23	A	2	...	1.6	2.0	2.6	2.6	2.4	2.5	2.3	1.4	80.9	...	80.6	79.8	79.8	79.8	80.0	80.0	80.0	80.0	80.0			
	B	14	5.1	5.3	6.6	5.5	5.0	4.5	4.3	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
13	A	3	...	1.4	1.7	2.1	2.1	1.7	1.7	1.6	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	11	1.8	2.5	3.4	3.4	3.3	3.1	3.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
24	A	1	...	1.7	2.1	2.6	2.6	2.4	2.5	2.3	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	17	5.8	7.7	8.1	8.4	8.0	7.4	7.1	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
15	A	8	...	1.3	1.8	2.1	2.1	1.8	1.8	1.7	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	10	10.2	11.1	12.5	11.4	10.9	10.4	10.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
16	A	5	...	1.4	1.7	2.1	2.1	1.8	1.8	1.7	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	9	11.0	13.3	14.6	14.8	14.3	13.4	13.7	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
17	A	3	...	1.3	1.6	2.0	2.0	1.7	1.7	1.6	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	10	12.3	14.6	15.1	15.1	14.8	14.4	13.8	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
18	A	5	...	1.4	1.7	2.1	2.1	1.8	1.8	1.7	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	10	12.3	14.6	15.1	15.1	14.8	14.4	13.8	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
19	A	9	...	1.4	1.7	2.1	2.1	1.8	1.8	1.7	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	10	10.6	11.1	12.4	12.8	12.3	11.5	11.1	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
20	A	11	18.8	20.8	22.8	22.3	20.0	16.5	15.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
	B	14	26.7	28.7	30.7	30.2	27.0	23.5	21.4	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
21	A	6	...	1.6	1.9	2.3	2.3	2.0	2.0	1.8	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	14	18.3	19.9	21.5	21.0	18.0	14.5	13.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
22	A	10	...	1.6	1.9	2.3	2.3	2.0	2.0	1.8	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	14	18.3	19.9	21.5	21.0	18.0	14.5	13.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
23	A	10	...	1.6	1.9	2.3	2.3	2.0	2.0	1.8	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	14	18.3	19.9	21.5	21.0	18.0	14.5	13.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
24	A	10	...	1.6	1.9	2.3	2.3	2.0	2.0	1.8	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	14	18.3	19.9	21.5	21.0	18.0	14.5	13.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				
25	A	10	...	1.6	1.9	2.3	2.3	2.0	2.0	1.8	...	80.3	...	80.2	79.0	79.0	79.0	79.0	79.0	79.0	79.0	79.0			
	B	14	18.3	19.9	21.5	21.0	18.0	14.5	13.0	...	...	80.3	79.3	78.0	78.0	78.3	78.6	78.9	78.9	78.3	78.3				

[illegible]



The distribution in percentage of water (fig. 13, 14, 15) is much more uniform. Just at flowering time there is considerable variation, due, doubtless, to exceedingly rapid changes taking place at that time.

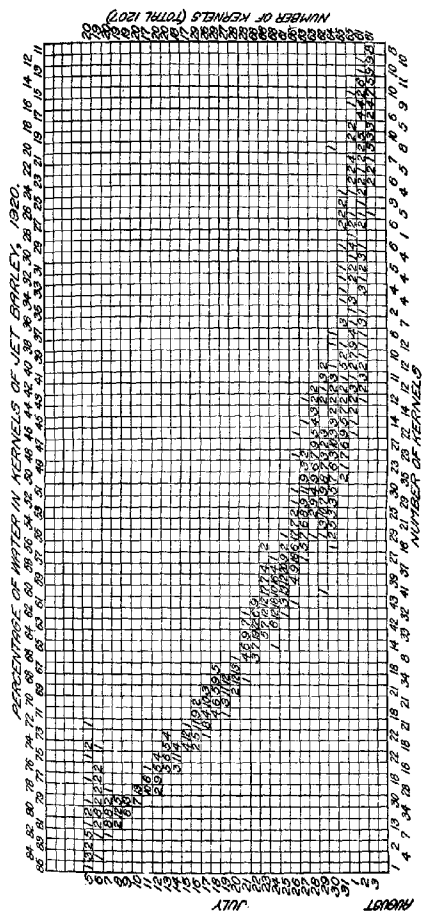


FIG. 13.—Distribution of kernels of Jet barley according to the percentage of water by days from flowering to maturity at Aberdeen, Idaho, in 1922.

Immediately following flowering the kernels show almost identical water content for any one day and almost identical daily decrease in water content for many days. The exact date of the beginning of spread in the distribution is difficult to determine. It is evident that it is not much before the seventeenth to twentieth day. By the twenty-third day



ripe kernels are in evidence. The changes apparently take place at a higher water percentage in the Baku than in the Jet variety. In the Baku the kernels dry very rapidly after they have reached the moisture

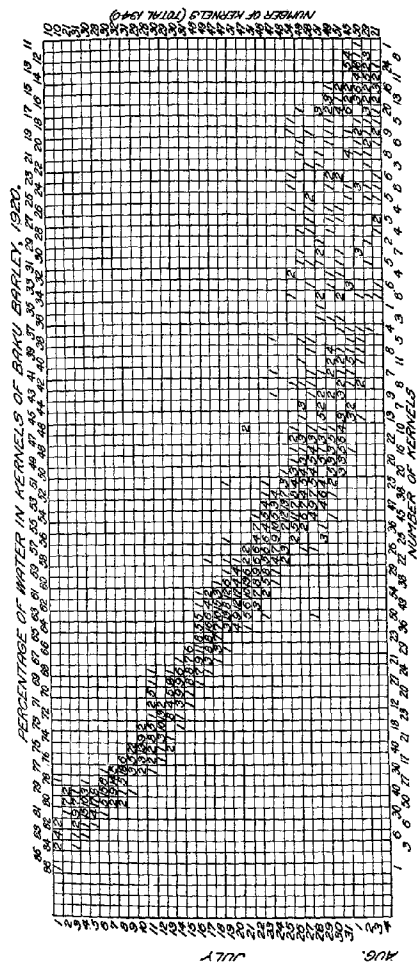


FIG. 15.—Distribution of kernels of Baku barley according to the percentage of water by days from flowering to maturity at Aberdeen, Idaho, in 1922.

content of 50 per cent. There are few kernels between 42 per cent and 50 per cent, showing that mechanical drying is taking place with great rapidity.

Thus far the data for the Jet and Baku varieties are quite comparable to those reported earlier. No conclusions can be drawn which were



not apparent in the previous studies. As has been pointed out, the sudden break in the curve of percentage of water, which has held a steady trend from flowering, could come about only through the abrupt termination of growth. It is not evident from these or previous figures whether this abrupt termination involves the whole kernel at once or whether there has been a final abrupt termination of activity in a limited area preceded by a gradual abandonment of tissue to the point where the active tissue remaining affords an insufficient volume in which to take care of the starch produced daily by the plant.

#### COLOR AS AN INDEX OF MATURITY

Although the figures, taken by themselves, do not afford sufficient basis for the analysis of the final phases of maturation, the color formed in the Jet variety offers a suggestive clue. The melanin-like pigment is apparently deposited only when activity has ceased. In the normal kernel the color appears just before final maturity. The earliest traces are found about the twentieth or twenty-first day after flowering. If a kernel is removed from the spike even six days after flowering, the black color will appear on drying. It seems reasonable to suppose then that when this color appears on the kernel, the section of the kernel immediately beneath it has ceased to function. That this assumption is plausible is evidenced by two related observations. The black color appears first on the dorsal surface just below the tip. The tip of the kernel up to this time consists of active tissue remaining from the ovary walls. As soon as the black color appears, this tissue withers, showing that the vascular system here has ceased to function. This peculiarity has been noted in other years, when it had been found possible to measure the true length of the kernel only after the withering of the tip. Previous to that time the measurement necessarily had to include tissues which had no real connection with the growth inside the nucellus. It was also in this region that cells showing early abandonment as active repositories for starch are most numerous, indicating that this region was the first to show lack of free movement of cell solutes.

The progress of color formation and loss of water, as evidenced by the denting and collapse of the kernels, is very definite. Traces of color appeared when the moisture content was about 62 per cent. It was present on most kernels by the time they had reached 60 per cent. The spot was very definitely marked when they had reached 58 per cent, and the area had spread well toward the end of the kernel when the water content had reached 56 per cent. Denting appeared at about 55 per cent and was common at 51 to 52 per cent. Color appeared on the ventral surface of the grain at 53 per cent and was on the ventral surface of most kernels by the time they had reached 48 per cent. The sides as well as the dorsal surface had begun to dent at about 50 per cent and were commonly dented at 46 per cent. The ventral surface was black with the exception of the furrow on some kernels at 46 per cent and commonly so at 43 per cent. Even the furrow was black in some instances at 42 per cent. Few furrows with traces of green were found under 40 per cent. Fine wrinkles appeared all over the kernel at percentages about 30 and on all kernels containing below 24 per cent of water.

When these color observations are considered with the data previously presented, they assist materially in the interpretation. The black area on the dorsal surface became marked about the twenty-second day after

flowering—that is, the average kernel was affected by this date. On the curve of dry weight (fig. 10), it can be seen that the rate of increase fell off markedly on this date and never again reached the rate held consistently for a long period previously. The water content began to drop very rapidly at this time, as did also the wet weight. It seems without question that the abandonment of tissue began actively when the water content of the average kernel had reached 58 per cent. On some kernels this change evidently was initiated at 62 per cent. Dry matter was added, however, to other parts of the kernel for several days. Growth was essentially complete when the color had covered the ventral surface. This occurred when the kernel had reached 46 per cent of moisture and was common on all kernels when they had reached 42 per cent. This 42 per cent coincides with the average date reported in various previous papers, and in this instance it is coincident with the average appearance of color. It would seem that, since 42 per cent represented the average water content when the spike was mature, this percentage would also represent the point at which the individual kernel should cease activity. That this is not true is due to the variation of spread before and after reaching maturity. Once a kernel has ceased active metabolism, the loss of water is very rapid and the spike average is lowered by wide range of water content in those kernels already mature.

While not all kernels showed the appearance of black at the same time, the earliest kernels exhibited the color when a water content of about 62 per cent was reached. From the small spot first occurring the color gradually spread over the dorsal surface of the kernel. The base of the kernel was last affected on both the dorsal and ventral surfaces. On the ventral surface, color first appeared on the flanks of the grain from the center of the kernel toward the tip. The green in the pericarp disappeared last from the furrow of the kernel and naturally this is the last active tissue. Shortly after the black color appears on the dorsal surface, the kernel begins to dent—that is, the loss of water in this region is sufficiently rapid to cause the tissue to collapse. The loss of volume at this time is apparent in the measurements of the grain. The kernels of all varieties of barley contract as they lose water in maturing. In the Jet variety the kernel collapses in a depression on the dorsal surface which reaches from the tip to the embryo. This is not common in barley varieties and doubtless comes about through the fact that in the Jet the tissues external to the nucellus are abnormally thickened in this region. Since such external tissues are not utilized for storage, the loss of water must be accompanied by an unusual reduction in volume. The final stage of ripening, or rather mechanical drying, is accompanied by a fine wrinkling of the entire surface of the kernel, as is common in other varieties.

Assuming that the kernels of all varieties ripen in the same way as those of the Jet, the data here presented add this much to our previous information in maturation. The process of ripening is abrupt, as has been previously apparent, but this final stoppage of activity is the culmination of a maturation which has already checked activity in some parts of the kernel. As may be seen in Figure 4, the drop in the average percentage of water is not accelerated by the abandonment of the limited portion of the kernels first affected. It is only when the black spot becomes common, indicating an average condition of abandonment, that the rate of loss of water is accelerated and the rate of deposit of dry matter checked. From this time there is a slight increase in average

dry matter. This increase doubtless is exaggerated in the averages due to the fact that all kernels are not equally advanced.

The date of maturation of individual kernels is partially dependent upon minor features of location. Supposing that a kernel can not be rejuvenated after the percentage of water has fallen below 44 per cent, the time of reaching this percentage may be postponed by protection from the sun and air. The loss of water from the kernel is active on hot days. There is no loss at night during growth. Very hot, dry, sunny days may lower the water of exposed kernels near maturity below the point of recovery. Those kernels well covered by the awns of other kernels ripen slowly. The kernels on the under side of the spike of nodding varieties ripen after those on the upper side. In varieties like the White Smyrna, where several kernels are inclosed in the leaf sheath, the kernels so inclosed ripen much later than the exposed kernels. Prolonged cool weather at ripening time must have a similar effect, and high yields of plump grain usually result from such ripening periods. These observations fit in with the appearance of the color in the Jet variety. While the amount of light reaching the kernel affects the intensity of the pigment and occasionally the time of its appearance, in general it affords an accurate index of the stage of maturity.

#### SUMMARY

In previous studies it has been shown that the average water content of the ovaries at flowering time is about 80 per cent and that the percentage of water in the growing kernel decreases uniformly day by day until the average for all the kernels of a spike is about 42, when all deposit of dry matter is interrupted and the kernels dry with great rapidity.

This 42 per cent does not represent the exact point where translocation of material becomes impossible but is lower than this point, as this is the average of all kernels on the spike and must include some with slightly more water which are still functioning and some with much less which ceased to function a day or two previously.

Endosperm cells mature abruptly; the proteid content probably reaches a density beyond which it can not function. The Jet is a naked variety in which a black pigment is formed in the pericarp whenever this tissue ceases to be active. The appearance of color indicates that the first region to mature is on the dorsal surface near the tip. The region of the embryo on the dorsal surface is still later, and the cells adjacent to the furrow on the ventral surface are the last to mature. The first cells on the dorsal surface are affected when the moisture content of the kernel has reached 60 to 62 per cent. The kernels are fully mature when the water has fallen to 46 per cent and have carried on only a limited amount of translocation for some time.

The date of final maturation can be postponed and the size of the kernel increased where kernels are protected by leaf sheaths or other shade and by cool weather at ripening.

## BACTERIAL LEAFSPOT OF GERANIUM IN THE EASTERN UNITED STATES<sup>1</sup>

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The florist's season for growing cuttings of geraniums (*Pelargonium* spp.) for garden and window-box planting is the season when this bacterial disease may be looked for, especially in crowded, ill-ventilated houses. Nearly every spring since 1912 and at any time during March, April, and May, according to the locality where the plants are grown, we have received diseased specimens. Very rarely has a diseased plant grown in the open been sent to us. Our first acquaintance with it, however, was at an earlier date when it occurred on the grounds of the United States Department of Agriculture in Washington (Pl. 1, A). In New Jersey and Maryland the disease has been especially prevalent and of a destructive nature, whole houses of rooted plants in some instances becoming unsuitable for market. It is a spot disease which makes the leaves unsightly and undermines the health and development of the cuttings. The year 1915 was evidently favorable for the disease, for it occurred very generally in the eastern part of the United States.

The spots are definitely outlined, usually irregular but occasionally circular in shape, and of a brown color. The spotting may begin either at the margin of the leaves or on the blade and may occur on either old or young leaves, although the older leaves or those nearest the soil are usually the worst affected. The youngest infected areas are watery looking, then become a reddish brown, later the tissue dries and becomes dark brown. When the disease is advanced the portion of the leaf between the spots turns dark and becomes dry and wrinkled. The spots, however, still show plainly in the dead area. The bacteria, which are motile, occur in great numbers in the spots. They are easily isolated from the young spots, as the epidermis can be sterilized with mercuric chlorid (1 to 1,000) for three to five minutes without much penetration.

Isolations were made from material received from Maryland (Pl. 1, B), and water suspensions of subcultures were sprayed on young geranium plants growing in the greenhouse. The plants were kept in a moist infection cage for two days. In another cage, which was also kept moist, were other geranium plants of the same age which were sprayed with sterile water. Typical spots appeared on the inoculated (sprayed) leaves in 9 days, and in 17 days the plants were badly spotted (Pl. 3, B). The controls did not show any spotting. Bacteria were abundant in the spots. The organism was reisolated and other geranium plants were infected with it by spraying. In four days spots began to show on the leaves of these sprayed plants. The temperature was higher at this time, being 80° F. during most of the daytime.

<sup>1</sup> Accepted for publication May 10, 1922.

Likewise, isolations were made from diseased material from New Jersey (Pl. 2). The same organism was obtained and typical spots produced in 17 days from the time of spraying the leaves with a water suspension of an agar subculture (Pl. 3, A). The organism was reisolated also from this New Jersey strain. The inoculations with the reisolated New Jersey organism were made in two different greenhouses, one in which the day temperature was 70° to 75° F. and another in which it was 55° to 60° F. The plants were sprayed and kept in infection cages for two days. In the warmer house infection occurred early and was well marked in 16 days, while there was but a trace of infection on the inoculated leaves in the cooler house. The disease did not progress in the latter case but continued in the former.

A bacterial disease of geranium plants was reported from Massachusetts<sup>2</sup> in 1898. The observers first found it on several different varieties during a season of rainy weather about the latter part of July on plants grown out of doors. The leaves were spotted and bacteria were found in the spots.

Another report of the disease from Massachusetts<sup>3</sup> stated that attempts had been made to isolate the organism but without success.

Still another report<sup>4</sup> said the disease had been noticed in that State every year for nine years. It was abundant and generally distributed, and gardeners had become concerned about it. The spotting was not serious in greenhouses, however, and it was thought to have been brought in from out-of-door stock.

Dr. Erwin F. Smith,<sup>5</sup> in volume 1 of his "Bacteria in Relation to Plant Diseases," mentioned the disease as one produced by stomatal infection and in volume 2 called it a disease of rainy seasons. In volume 2 he also stated that the organism was isolated in his laboratory and the disease reproduced on geranium leaves by John R. Johnston, inoculating from pure cultures. No further work on this disease was done by Mr. Johnston or by Dr. Smith beyond recognizing it as a yellow organism with a polar flagellum. He states to me that none of the earlier isolations from the Washington material ever greened the medium.

A bacterial disease of the leaves of *Erodium* and *Pelargonium* in Texas was described in *Phytopathology*<sup>6</sup> for August, 1914. The general appearance of the spots on the leaves in the illustrations of the article led us at first to think that the Texas disease described was the same as the one our laboratory had been dealing with, although Lewis's figures 2 and 3 indicate a much more active parasite. When comparisons of cultural tests were made and what seemed to be important differences were noted, it was decided that the two organisms must be different and that these different features would be accentuated if worked out in more detail. That has been done, and careful observation and repeated tests have established the writer's belief that the organisms are not the same, for in cultural tests important differences persist. Perhaps Mr. Lewis's work was done with the *Erodium* strain and the host may be responsible for these differences. He does not state in his paper which isolation he

<sup>2</sup> STONE, George E. and SMITH, Ralph E. A DISEASE OF THE CULTIVATED GERANIUM. *In* Mass. Agr. Exp. Sta. 10th Ann. Rpt., 1897, p. 67, t. pl. 1898.

<sup>3</sup> A GERANIUM DISEASE. *In* Mass. Agr. Exp. Sta. 12th Ann. Rpt., 1899, p. 57-58, 1900.

<sup>4</sup> STONE, G. E., and MONAHAN, N. F. BACTERIOSIS OF GERANIUMS. *In* Mass. Exp. Sta. 19th Ann. Rpt., p. 164, 1907.

<sup>5</sup> SMITH, ERWIN F. BACTERIA IN RELATION TO PLANT DISEASES. V. 1, p. 92, 1905; V. 2, p. 39, 61, 1911.

Washington, D. C. Carnegie Inst. Publ. 37.

<sup>6</sup> LEWIS, I. M. A BACTERIAL DISEASE OF ERODIUM AND PELARGONIUM. *In* *Phytopathology*, v. 4, no. 4, p. 221-232, pl. 10, 1914.

used for his cultural tests. He states that he isolated an organism from both *Erodium* and *Pelargonium* and that he was convinced that the organism from the wild *Erodium* and the cultivated species of geranium (*Pelargonium*) were one and the same. He found that both strains cross-inoculated readily but does not mention making any comparative cultural tests of the two strains. In all probability he used one strain only for his cultural tests.

The comparisons in this paper were started with the idea of finding enough agreement in the cultural tests to establish the identity of our organism as *Bacterium erodii*, and with that in view we cast about for explanations to account for the differences. One striking difference between the Texas and Maryland organisms is in the production by the Texas organism of a green fluorescence in beef agar, beef bouillon, sterile milk, and various other media. Very careful observations were made, but no trace of green fluorescence could be detected at any time in the tests with the Maryland or New Jersey isolations. The continued comparisons in this paper were made with the New Jersey isolation. Morphologically the Texas and New Jersey organisms are much alike and in some of the cultural tests are identical. Although in two to five days we have never obtained leaf-spots such as Lewis figures the two organisms produce somewhat the same type of disease on geranium leaves apparently under the same conditions, yet if they are the same organism why does one produce green pigment and the other not?

Thinking the green color might have formed through some particular property of the medium, two lots of peptone-beef bouillon (beef infusion) were made in which Witte's and Difco peptone were used as well as two lots of beef extract media with the two kinds of peptone. Our organism acted the same in all in respect to color—there was not a trace of greening. Then Mr. Lewis's platings from diseased material were considered. At the start an organism appeared on his plates which greened the agar. Subcultures from these colonies picked from the plate produced the disease. Could this pigment formation be the individuality of a strain? If so, it is a striking variation and a feature that must be reckoned with when comparing strains and varieties in proving up a new organism.

The New Jersey strain of the geranium leafspot disease was used for the tests described hereafter in this paper. To facilitate comparison with *Bacterium erodii* the same order is followed here as in the article by Lewis describing that organism.<sup>1</sup> No work was done with *Bacterium erodii* itself, as we had no culture of that organism nor were we able to obtain Texas material for our own isolations. The comparisons, therefore, were all made with the rather full tests published by Mr. Lewis in the article just cited.

#### CULTURAL CHARACTERISTICS OF THE ORGANISM FROM THE DISTRICT OF COLUMBIA, MARYLAND, AND NEW JERSEY

**AGAR PLATES.**—On beef infusion peptone agar + 16.5 plating from a 2-day-old bouillon culture, colonies do not appear until the third day. In reflected light they are cream color, shining, round with a smooth surface. In transmitted light under a hand lens they are a cream color in the center and bluish outside of center. There are delicate reticulate

<sup>1</sup> Lewis, I. M. op. cit.

markings in the interior; there is no zoning, but a marginal ring occurs on some colonies after they are up several days. Colonies are 2 to 4 mm. in diameter. The agar does not change color. (Unlike the Lewis organism.)

**AGAR STROKE.**—In 2 days there is a translucent wet shining rather thin growth on +13 peptone-beef infusion agar. In 3 days the growth is cream colored with undulating surface. There is no green color in the agar or condensation water. The surface is finely pitted at 4 days; crystals form in from 2 to 10 days. There is no viscosity until cultures are 1 month old or older, but even then there is no green color. (The Lewis organism produces a green pigment.)

**GLUCOSE AGAR STAB.**—This is a favorable medium. Growth is fairly rapid and abundant on the surface of the stab; scant along the line of puncture. The color of 4-day-old cultures is Ridgway's Naples yellow,<sup>1</sup> and this is a deeper color than the growth on plain beef agar. (Same as the Lewis organism.)

**LACTOSE AGAR STAB.**—In this medium there is growth with abundant crystals. No green color. (The Lewis organism produces a green color.)

**STEAMED POTATO CYLINDERS.**—A thin yellow growth occurs on the potato in 24 hours. In 2 days it is a Naples yellow; in 3 days the potato begins to darken a little, but there is no green fluorescence. The potato cylinders are still firm after a month. The starch in the cultures gives a purple reaction when tested with iodine in potassium iodide. (Lewis's organism produces a green fluorescent pigment after 48 hours.)

**STEAMED COCONUT.**—A thin faintly yellow growth occurs in 2 days on pieces of coconut steamed in tubes. Growth takes place in the water also but is not a yellow color. In 2 weeks the growth on the surface of the coconut is still thin and not viscid. (The Lewis organism produces a viscid growth on this medium.)

**LITMUS MILK.**—There is a trace of clearing (whey) in 3 days but no color change. In 6 days there is a faint bluing in bands and a clearing at the surface for nearly 1 cm., but no coagulation. In from 10 to 15 days the bands of color are faint and clearing has taken place in from one-half to the entire tube. Then it is a reddish blue color, dark hyssop violet, according to Ridgway. (With Lewis's organism the liquid is all a clear yellowish color in 8 days.)

**STERILE MILK.**—No clearing occurs before 5 days even at the optimum temperature of 26° to 28° C. At that time there is a ring of whey, cream colored, 3 to 5 mm. deep. Coagulation comes soon after the clearing appears; the curd is soft. There is no color change. Tested at 17 days the hydrogen-ion concentration expressed in  $P_H$  value is 6.4 (Brown cresol purple). In 30 days over half the curd has been digested. (The Lewis organism does not coagulate sterile milk; it produces green fluorescence with age.)

**DUNHAM'S SOLUTION.**—There is heavy clouding in this medium in 48 hours, but not any marked difference from peptone bouillon. (Like the Lewis organism.)

**PEPTONE BOUILLON.**—The organism clouds peptone beef infusion bouillon +10 to +15 in 24 hours at a temperature of 23° to 27° C., when transfers are made from a young fluid culture. At 18° to 20° it is not clouded until 48 hours. Clouding is heavy in 2 days at 27°. Pseudo-

<sup>1</sup> RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

zooglea are present. In 2 to 4 days an incomplete pellicle is formed and the medium is clear below the pellicle. Cultures are not viscid until 1 month old or older. When the tube is agitated the pellicle drops to the bottom in flocks. No green color is produced in either young or old cultures. Some bouillon was made containing Witte's peptone, and the organism grown in it was compared with that containing Difco peptone to see whether the green color would appear. It did not, nor when extract of beef was used with the two kinds of peptone instead of the beef stock. There was no trace of a green color at any time. (The Lewis organism greens the bouillon.)

**DUNHAM'S SOLUTION WITH METHYLENE BLUE.**—The organism grows well in Dunham's solution colored with methylene blue. The color changes to a faint blue in four days. This test was repeated with the same result. The color reduced from the bottom of the tube upward. In 22 days the methylene blue is reduced to a mere trace. In 1 month after inoculating the color begins to return, but is a green instead of blue—light hellebore green, according to Ridgway.<sup>9</sup> (The Lewis organism does not change the blue color; observations were made over a period of 4 weeks.)

**BOUILLON WITH SODIUM CHLORID.**—Growth takes place in neutral beef bouillon to which 2.5 per cent sodium chlorid is added. Tests were made with 3.5 and 4 per cent sodium chlorid, but no growth took place in either of the latter media. (Like the Lewis organism.)

**BOUILLON OVER CHLOROFORM.**—No growth occurs in 10 cc. of peptone-beef bouillon over 5 cc. of chloroform. Three different tests were made, one in which the quantity of chloroform was reduced to 3 cc. No growth occurred. The same bouillon inoculated without chloroform showed abundant growth in 48 hours. (The Lewis organism grows in this medium.)

**DECOCTION OF GERANIUM LEAVES.**—Fifty gm. of fresh geranium leaves were boiled in a liter of water, then filtered and autoclaved. It tested +6, Fuller's scale;  $P_H$  4.1. The organism was slow in appearing and grew but feebly in this medium. (Not unlike the Lewis organism.)

**GERANIUM AGAR.**—Some of the decoction described above was made up into agar by adding 2 per cent agar. The medium titrated +6 Fuller's scale;  $P_H$  4.1. A mere trace of growth occurred in the agar made from the decoction. (Not unlike the Lewis organism.)

**NITRATE BOUILLON.**—There is slight growth in 2 days in beef bouillon containing 1 per cent potassium nitrate; good growth in four days. A partial pellicle is formed rather heavy with crystals. This pellicle falls apart in handling the tube. (The Lewis organism makes a persistent pellicle.)

**FERMI'S SOLUTION.**—In 2 days there is a faint clouding at temperatures of 25° to 28° C. The clouding is still faint in 2 weeks. In older cultures an incomplete pellicle is formed. There is no green fluorescence with age. A culture 3 months old is Ridgway's old gold color. (The Lewis organism produces green fluorescence.)

**COHN'S SOLUTION.**—There is no growth in Cohn's solution. (The Lewis organism grows in Cohn's solution without greening.)

**USCHINSKY'S SOLUTION.**—At temperatures 25° to 28° C., which are favorable temperatures for this organism, there is only a mere trace of growth in 2 days, and no heavier at 7 days. In 2 weeks clouding is better,

<sup>9</sup> RIDGWAY, Robert, *OP. CIT.*



and later an incomplete pellicle forms. There is no change in the color of the medium when cultures are 3 months old. (The Lewis organism grows promptly in this medium without greening.)

**GELATIN STAB.**—With +10 beef-infusion gelatin, stab and plates, the liquefaction occurs slowly. Plates thickly sown show slight liquefaction in 7 days, and most of the plate liquefies in 12 days. Temperature 18.5° to 20° C. The liquefaction starts in the stab cultures in 6 days and continues slowly across the surface. In 1 month they are about one-fourth liquefied. In 2½ months the stabs are slightly over half liquefied. In 3½ months one stab was entirely liquefied and the others three-fourths. In 4½ months all cultures are entirely liquefied. (The Lewis organism liquefies a stab culture in 4 weeks.)

**LACTOSE LITMUS AGAR.**—Growth takes place readily. There is no color change in 5 days. In 7 days the bacteria have taken up color, and a mass on a platinum loop looks green. The slant has become blue, but the agar at the bottom of the tube has not changed color. In 16 days the color of slant is still blue; that part of the medium in the bottom of the tube is unchanged. (The Lewis organism reddens the medium.)

**STEAMED CARROT CYLINDERS.**—Growth does not take place quickly on carrot cylinders, but in 12 days the surface of each is covered with a creamy growth, smooth, wet shining, not viscid. There is no browning of the medium in 25 days. (The Lewis organism browns the medium.)

**STEAMED WHITE TURNIP.**—Growth is slow in starting on this medium and is always thin. It is creamy in color, wet shining, not viscid. The medium is neither softened nor browned in 25 days. (Mr. Lewis says this medium is favorable for growth and that it becomes soft and brown.)

**INDOL.**—There is a slight production of indol in Dunham's solution cultures 10 days old. It is still slight when the cultures are 18 days old. The tests were made with sulphuric acid and sodium nitrite. (Like the Lewis organism.)

**HYDROGEN SULPHID.**—Hydrogen sulphid is produced. The organism was grown on potato cylinders, beef agar, lactose agar, and in beef bouillon. The test was made by suspending lead acetate paper in the culture tubes. The paper became well blackened in every case. (The Lewis organism does not produce hydrogen sulphid in any of these media, not even after prolonged exposure.)

**AMMONIA PRODUCTION.**—The organism produces ammonia. Cultures of beef bouillon and peptone water both 10 days and 3 weeks old were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. The cultures were then heated in a water bath. A red-brown color appeared on the filter paper immediately. (Same as Lewis's organism.)

**NITRATE REDUCTION.**—There is no reduction of nitrates to nitrites. Tests were made with nitrate bouillon in which the organism grew very well. Ten-day and 28-day cultures were tested. *Bacillus coli* grown in the same medium and tested by the same method (starch-iodin sulphuric acid test) gave a positive test. (Same as Lewis's organism.)

**REDUCTION OF LITMUS.**—Litmus is reduced in 10 to 15 days in sterile milk. (Not appreciably different from Lewis's organism.)

**METHYLENE BLUE.**—Reduction of methylene blue takes place in 7 to 11 days, according to temperature. Tests for the reduction were made in milk colored to a robin's egg blue. The reduction begins from the bottom of the tube and goes upward. In 3 days it is white at the bottom of the tube for one-eighth of the liquid. The rest of the liquid is a slightly lighter blue than the control. No coagulation occurs. In 11 days there is entire reduction in some tubes. When entirely reduced the milk coagulates. Those tubes with a slight blue at the surface are not coagulated. In 15 days the blue in all tubes is wholly reduced. Temperature 20° to 22° C. When tubes of methylene blue milk are inoculated and placed at 27° to 28° reduction occurs throughout in 6 days. (With the Lewis organism the tests for the reduction of methylene blue were negative.)

#### THERMAL RELATIONS

**THERMAL DEATH POINT.**—The thermal death point lies between 51° and 51.5° C. Transfers were made from well-clouded 24-hour-old cultures and tested many times. The beef bouillon titrating +13 to +17 on Fuller's scale ( $P_H$  6.8 to 7.0) was held in thin-walled test tubes and after transfers were made was kept and heated for 10 minutes at constant temperature in a water bath. Growth occurred at all temperatures tried (48°, 48.5°, 49°, 49.5°, 50°, 50.5°, 51°) except 51.5°. Once in six tubes there was no growth at 50°, but in another six tubes all grew at 50.5°. In one out of two tests growth took place at 51°. None at 51.5°. (The thermal death point of the Lewis organism is 48.5°.)

**OPTIMUM TEMPERATURE.**—The optimum temperature is around 27° C. Tests were made with temperatures from 10° to 40°. The organism clouds +15 bouillon in 24 hours from 23° to 28°, but in 48 hours the growth is heavier at 26° to 28°. (Agrees with the Lewis organism.)

**MINIMUM TEMPERATURE.**—Growth occurs at 1° C. In one test with the organism grown in +15 beef bouillon there was a trace of clouding in 16 days. The temperature ranged from -1° to 1.75°. In a second test in which the thermostat ranged from 0.6° to 1.25°—never over 1.25° and usually under 1°—growth was visible in 49 days. It was never more than a faint clouding. (The Lewis organism grows in both beef agar and beef bouillon at 0°.)

**MAXIMUM TEMPERATURE.**—Growth occurs at 35° C, but it is very feeble. There is none at all at 36° to 37°. (Very little difference from the Lewis organism.)

#### TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. Agar and bouillon transfers were placed in a specially devised jar from which the oxygen was removed as follows: A solution of 35 gm. of potassium hydroxid dissolved in 350 cc. of water was poured over 40 gm. of pyrogalllic acid. The bottle for this solution had previously been adjusted in a jar where the transfers were. The mixture was poured in the bottle and its top left uncovered; but the jar was covered, then another cover inserted in a bed of mercury was placed over the whole. A tube of methylene blue milk and litmus milk had been placed in the jar with the cultures as controls on the presence of oxygen.

No growth occurred in any of the cultures in the jar. The bouillon control in the room showed good growth. The methylene blue had faded considerably in 11 days. The litmus did not fade until 15 days after setting up the experiment. A careful watch was kept for over 3 weeks, but no growth took place in the cultures until they were removed from the jar. (Like the Lewis organism.)

#### RELATION TO MOISTURE

This test was followed out according to Mr. Lewis's rod method. Glass rods were held in place in test tubes by passing them through the cotton plugs, after which the tubes were sterilized. Next the rods were dipped to a uniform depth in a 6-day-old bouillon culture, returned to the tubes, and left to dry at room temperature which varied from 18° to 23° C. Care was taken that the rod did not rest against the side of the tube and prevent a uniform drying. At intervals of 24 hours several of these rods were transferred each to a tube of beef bouillon. Growth occurred in one test after the cultures on rods had been dried 7 days. In a second test made exactly the same way, growth did not occur when dried 7 days but did occur at 6 days. Evidently 6 days is about the limit of drying for a 6-day-old culture of this organism. (Like Lewis's organism.)

#### GROWTH IN FERMENTATION TUBES

**GAS FORMATION.**—The organism is aerobic so far as tested and does not form gas. It was tested in fermentation tubes in the presence of each of the following carbon compounds: saccharose, dextrose, lactose, maltose, and glycerin; 1 per cent of these being added to a 2 per cent water solution of Witte's peptone. No gas formed in any of the tubes. Growth occurred in the open arm of each tube but none in the closed arm.

The cultures were tested for acidity after they had grown 21 days. Five tests were made—two in January, in which Witte's peptone was used in one and Difco peptone was used in the other. Another test containing Witte's peptone was made in June. The fourth and fifth tests were made in July with Witte's and Difco peptone, as shown by Table I. Tests 1, 2, and 3 are with Witte's peptone, 4 and 5 with Difco. The results are as indicated in Table I, and it is interesting to note how the same organism varies. With Difco peptone, the acidity of the medium was considerably reduced by the growing organism.

Titration with phenolphthalein were made before inoculating and again after the organism had grown 21 days. The first test after 21 days showed there was little change in acidity in the cultures. The saccharose, dextrose, and glycerin cultures were slightly more acid than the controls; the maltose and lactose cultures were less acid. In the second test after 21 days all cultures were more acid than the controls. In the third test after 21 days there was no change in acidity with dextrose, lactose, and glycerin; there was increase with saccharose and decrease with maltose. The fourth and fifth tests after 21 days were made with Difco peptone. Titrations showed there was decidedly less acidity in the cultures than in the uninoculated media.

To determine further the changes in acidity, some of each of the same medium was tested with the indicators brom thymol blue, brom cresol purple, phenol red or cresol red, before inoculating. This was done at the same time they were tested on Fuller's scale. In all five tests the

$P_H$  values were found to vary from  $P_H$  7.0 to 6.6—that is, from neutrality slightly to the acid side. After inoculating, vigorous growth occurred in all cultures. At the end of 21 days tests were again made with the indicators. There was no increase in the hydrogen-ion concentration. As indicated by the records ( $P_H$  7.2 to 8.6) there was decrease in the hydrogen-ion concentration. In all five tests there was a uniformity of these changes in acidity, as shown by the indicators named above.

The titrations with phenolphthalein of cultures grown in carbohydrate media containing Witte's peptone were somewhat different from those made by Mr. Lewis with his organism, though not so strikingly so as with the Difco peptone. The second test was the only one that showed an increase in acidity throughout.

At the time of Mr. Lewis's experiments (his work was published in 1914) Witte's peptone was in general use in laboratory media.

TABLE I.—Acidity of cultures of the writer's geranium leafspot organism

Medium.	$P_H$ .					Fuller's scale.				
	Witte's peptone.			Difco peptone.		Witte's peptone.			Difco peptone.	
	First test.	Second test.	Third test.	Fourth test.	Fifth test.	First test.	Second test.	Third test.	Fourth test.	Fifth test.
Saccharose:										
Uninoculated.....	7.0				6.8	+8.5	+9	+10	+10	+13
Inoculated 21 days...	7.6	7.6	7.0	7.0	8.2	+12	+17	+15	0	+11
Dextrose:										
Uninoculated.....	6.8	6.7	6.8	6.8	6.6	+8.0	+10	+10	+11	+13
Inoculated 21 days...	7.6	7.8	7.4	7.4	7.4	+16.5	+21	+10	+6	+8
Maltose:										
Uninoculated.....	6.9	6.8	6.8	7.0	6.6	+8.5	+9	+9	+11	+12
Inoculated 21 days...	8.2	8.0	7.4	8.4	7.4	+4	+16	+7	-5	+8
Lactose:										
Uninoculated.....	6.8	6.8	6.8	7.0	6.6	+9.5	+9	+9	+10	+12
Inoculated 21 days...	8.2	7.6	7.4	8.6	8.0	+3	+18	+9	-5	+8
Glycerine:										
Uninoculated.....	6.9	7.0	7.0	7.0	6.8	+8.0	+8	+9	+11	+14
Inoculated 21 days...	8.1	7.6	7.4	7.6	7.4	+10.5	+15	+9	+3	+9
Peptone water:										
Uninoculated.....	7.0	No test.		7.0	6.8	+9.0	No test.	+9	+9	+12
Inoculated 21 days...	8.1	...do...	7.4	8.4	7.4	+8	...do...	+7	-5	+8

#### SENSITIVENESS TO ACIDS AND ALKALIES

In uncorrected beef juice (titrating +23) part made alkaline with sodium hydroxid and part acidulated with hydrochloric acid the titration range of the organism is -6 to +28 ( $P_H$  8.7 to 5.7).

Table I shows there is a reduction in the H-ion concentration after the organism has grown on the media 21 days when both Witte's and Difco peptone are used. A reduction of acid is indicated on Fuller's scale when Difco peptone is used, while with the Witte's peptone there is no uniformity of reduction or increase of acidity (indicated on Fuller's scale) though the table shows there are more cases of increase than decrease.

The Lewis organism increased in acidity from 4 to 8 points on Fuller's scale as Table II shows.

TABLE II.—Acidity of Lewis's geranium leafspot organism

Medium.		Fuller's scale.	Increase in acidity.
Saccharose.....	Uninoculated.....	+10	8
	Inoculated 21 days..	+18	
Dextrose.....	Uninoculated.....	+10	7
	Inoculated 21 days..	+17	
Maltose.....	Uninoculated.....	+8	4
	Inoculated 21 days..	+12	
Lactose.....	Uninoculated.....	+8	8
	Inoculated 21 days..	+16	
Glycerine.....	Uninoculated.....	+10	6
	Inoculated 21 days..	+16	

In neutral beef bouillon acidulated to +22 with hydrochloric acid there is no growth. This test was made because it was not stated in Mr. Lewis's article what his foundation beef bouillon was. In the same neutral bouillon acidulated with malic acid there is growth at +10 ( $P_H$  7.1); none at +19.5 ( $P_H$  6.5). Acidulated with lactic acid, there is growth at +9 ( $P_H$  7.1). There is none at +22 ( $P_H$  6.1).

Thinking that perhaps Mr. Lewis had used beef extract and Witte's peptone instead of beef infusion and Difco peptone, tests were made using beef extract media with these acids and with tartaric acid added to the list.

There was growth in the extract (uncorrected +7) made neutral and then acidulated with malic acid to +10 ( $P_H$  6.2); no growth in +21 ( $P_H$  4.9).

With lactic acid there was growth in +9 ( $P_H$  6.4); none in +18 ( $P_H$  4.9).

With tartaric acid there was growth in +9 ( $P_H$  6.4); none in +20 ( $P_H$  4.7).

With hydrochloric acid there was no growth at +21 ( $P_H$  4.0).

In peptonized beef juice made alkaline with sodium hydroxid, growth takes place to -6 ( $P_H$  8.7). No growth takes place at -9 ( $P_H$  9.1).

With peptonized extract of beef, the titration of which was +7 reduced with sodium hydroxid to -2, -6.5, and -13.5, there was growth at -2 and -6.5 ( $P_H$  8.4 and 9.4, respectively).

In a long series of tests with peptone-beef bouillon in which the beef juice was used, the titration range of the organism was found to be -6 to +28 and the  $P_H$  range 8.7 to 5.7.

Mr. Lewis's notes are as follows regarding sensitiveness to acids and alkalies:

The organism grows best in culture media that are acidulated to +15 or +20 with HCl. Growth does not occur at +40 and is retarded above +30. The same degree of acidity is not tolerated when malic, tartaric or lactic acids are employed. The growth in +8.5 lactic acid appears to be about the optimum for this acid, and the maximum is lower than for hydrochloric. Growth fails at +30 tartaric and malic.

In media titrated to -10 with normal NaOH the growth is retarded, while no growth occurs at -20. In neutral bouillon growth proceeds more slowly than in +10 or +20.

## MORPHOLOGY OF THE ORGANISM

The organism was studied in the cells of the spots on the leaves which had been sectioned and stained with carbol fuchsin and in 2-day-old beef agar cultures stained with carbol fuchsin. It is a short rod with rounded ends; stained in diseased material it is  $0.62\ \mu$  to  $1.46\ \mu$  long and  $0.61\ \mu$  to  $0.83\ \mu$  wide. In the agar cultures the size is  $0.62$  to  $1.25\ \mu$  long and  $0.41\ \mu$  to  $1.04\ \mu$  wide. In cultures the rods hang together in chains of 2 to 16 elements, but mostly 6 to 8. Capsules were demonstrated in 2-day-old agar cultures stained with Ribbert's capsule stain and also with carbol fuchsin.

Flagella were stained with Casares-Gil's flagella stain. There is one flagellum at a pole, rarely one at each pole. One case was observed of a branched flagellum at a single pole. No spores were demonstrated in a culture of any age nor were any involution forms seen. The organism is not acid fast nor does it stain by Gram's method but is stained readily with ordinary basic aniline stains.

The morphological differences between the two organisms are slight. The size of the Lewis organism is  $1.2$  to  $1.8\ \mu$  by  $0.6$  to  $0.8\ \mu$ , with rare involution forms. No spores or capsules are demonstrated. It is Gram negative, not acid fast, and bears 1 to 3 polar flagella at one pole only.

## NATURAL INFECTION AND CONTROL

The disease under the writer's observations is one that occurs throughout the Eastern States in greenhouses and attacks the plants usually when they are rooted cuttings and growth is being forced. We have never noticed the disease on old, slow-growing plants except in a few cases when the plant had been cured of the disease and then the spots were few and on leaves that had held over from the early attack. When the disease occurs out of doors, as it sometimes does, it is due to crowding or to unfavorable weather conditions acting on susceptible varieties. The occurrence of the disease on the grounds of the United States Department of Agriculture was mentioned in the early part of this paper. It is probable that the disease began in the greenhouse unobserved and continued when the plants were set out in the beds, hot weather and moist conditions favoring a rapid development of the disease. It has not occurred in the grounds for the last 10 years, the very susceptible varieties having been discarded.

There has never been any insect present on any of the diseased material received from the various sources. Occasionally geranium leaves are received which are infested with red spider (*Tetranychus telarius* L.), but the spotting due to this mite is of a different type.

From all the evidence gathered the organism seems to be one harbored in the soil. The disease is not a serious one unless the physical condition of the plant is weakened by too rapid growth and too moist or too warm an atmosphere with little chance of air circulating between plants, and last, but not least, too little care in watering.

Experiments leading to the control of the disease could not be undertaken in the greenhouses where the disease occurred because of distance. Control work, however, was attempted with our own plants on which the disease had been produced. The spotted leaves were picked off, the plants separated from each other in order to give them light and

plenty of air, and care was exercised in watering them. Under these conditions the disease disappeared entirely by the end of six weeks and did not return.

#### TECHNICAL DESCRIPTION OF THE ORGANISM

##### *Bacterium pelargoni*, n. sp.

A motile rod with rounded ends, usually borne in pairs, occurring also in chains average size 1.02 by 0.67  $\mu$ ; one polar flagellum; capsules; no spores or involution forms noted; agar colonies cream colored, round, shining, with delicate internal markings; liquefies gelatin slowly; reduces litmus and methylene blue and greens the latter; produces ammonia, indol (slight) and hydrogen sulphid; does not reduce nitrates to nitrites; grows weakly in Uschinsky's and Fermi's solutions and not at all in Cohn's; coagulates sterile milk; is aerobic; has feeble diastasic action on potato starch; maximum temperature 35° C., minimum 1°, optimum 27°; thermal death point between 51° and 51.5°; resists drying six days when 6-day-old cultures are used; tolerates sodium hydroxid in peptone-beef infusion to -6 Fuller's scale (colorimetric determination  $P_H$  8.7) and hydrochloric acid to +28 ( $P_H$  5.7); does not produce gas from sugars or alcohols tested; is Gram negative; not acid fast; stains readily with carbol fuchsin, methylene blue, and gentian violet; is pathogenic to cultivated geraniums, causing dead spots on the leaves.

The organism is unlike the one described by Lewis from Texas as the cause of a spot disease of *Erodium* and *Pelargonium* and may be called *Bacterium pelargoni*. It has been reported from various parts of the Eastern United States from Virginia to Massachusetts and has been under observation in the Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture, at different times for the last 20 years.

#### SUMMARY

A bacterial leafspot disease of the cultivated geranium occurs widespread in the Eastern States. It is mostly a greenhouse disease but occurs occasionally on plants grown out of doors.

The organism was isolated from diseased plants received from different sources and the disease reproduced on the leaves of healthy plants.

Warm, moist conditions with poor ventilation are necessary for the organism to infect the leaves extensively.

Care in regulating the temperature, air, and moisture conditions of the greenhouse and in giving plenty of space to plants grown out of doors will go far toward preventing the appearance of the disease and toward curing it when it is present. All spotted leaves should be removed and destroyed. Very sensitive varieties should be discarded.

The name *Bacterium pelargoni* is suggested for the organism causing the disease.





**PLATE 1**

- A.—Bacterial spots occurring on plants in Department of Agriculture grounds,  
Washington, D. C.  
B.—Bacterial spots occurring on greenhouse plants in Maryland. Natural size.

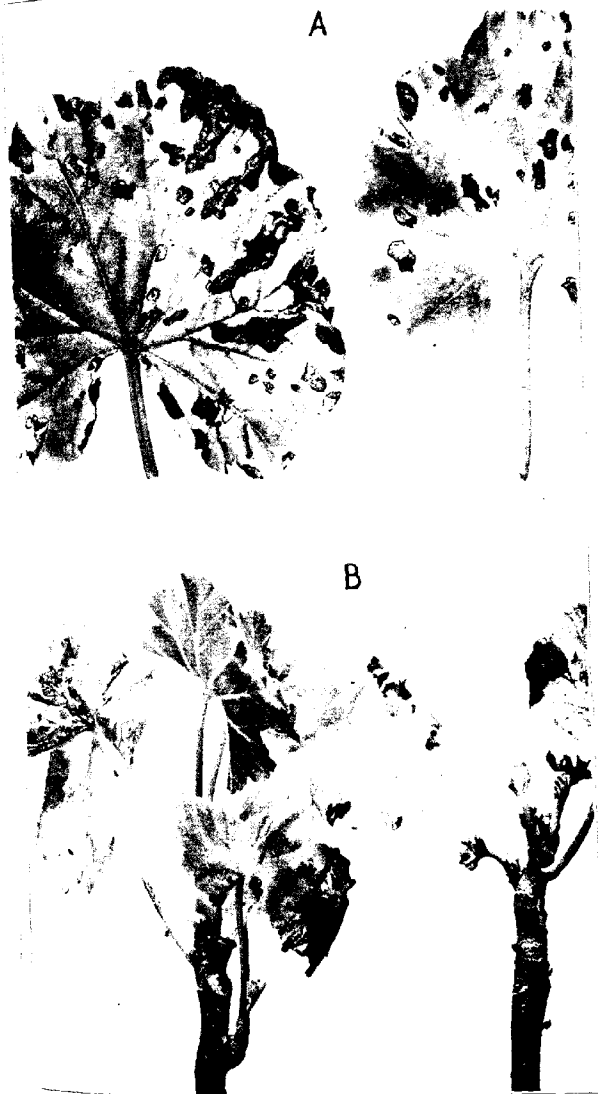




PLATE 2

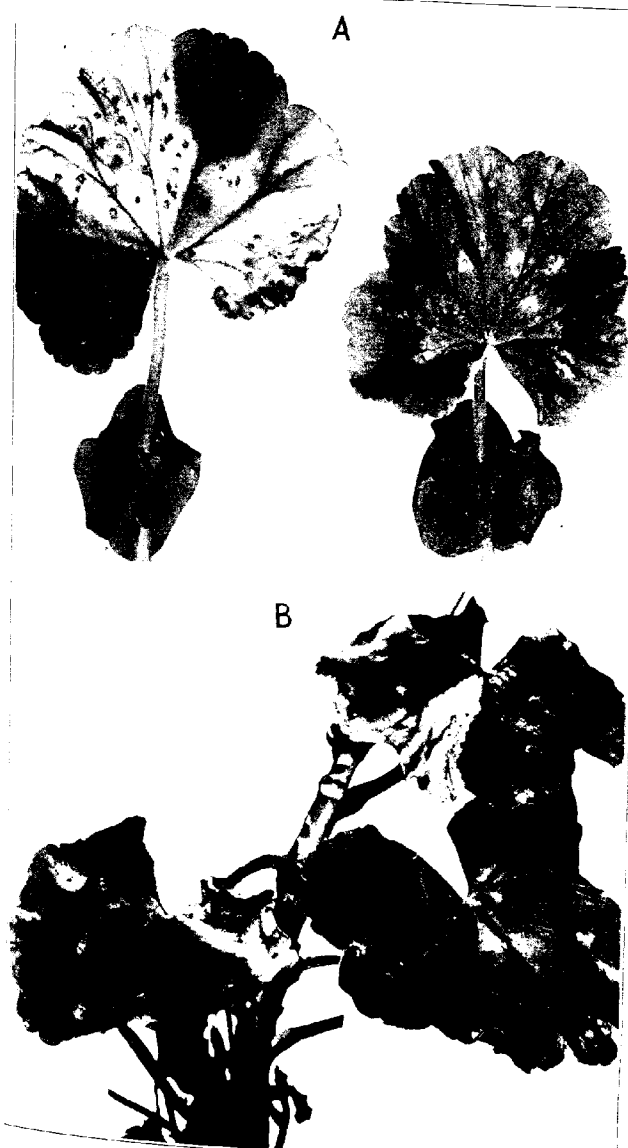
Geranium leafspot from New Jersey. Natural size.

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**PLATE 3**

A.—Geranium leaves inoculated with leafspot organism (New Jersey) by spraying.  
May 5, 1920. Photographed May 26, 1920.

B.—Geranium leaves sprayed with water suspension of geranium leafspot organism  
(Maryland) March 31, 1915. Photographed April 25, 1915.  
Natural size.





# HYDROGEN-ION CONCENTRATION AND VARIETAL RESISTANCE OF WHEAT TO STEMRUST AND OTHER DISEASES<sup>1</sup>

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## INTRODUCTION

The historical development of the subject of varietal resistance to disease among plants has been traced in considerable detail by a number of writers (4, 9, 21, 29, 31),<sup>3</sup> so that a brief summary of the many theories proposed in the literature will suffice to give the background for the present study. Among the morphological characters which have been considered responsible for resistance to some parasite are size of stomata, waxiness or hairiness of leaves or stems, toughness or thickness of cell walls or cuticle, and structure and extent of root system. Among the physiological characters are the ability to form cork around the invading organism; such rapid growth of the host that the parasite is unable to keep up with the growing tip; the presence of phagocytes or antitoxins in the host (conferring immunity in a manner analogous to that of animals); the activity of enzymes; the concentration of the cell sap; the lack of any substance stimulating the chemotropic responses of the organism necessary for the latter's successful entry and progress in the susceptible variety; the lack or subminimal amount of some nutrient necessary to the growth of the parasite in the host tissue; the presence in lethal quantity of some substance toxic to the invading organism. In support of these various views comparative determinations of many plant constituents, among them silica, manganese, cellulose, starch, sugar, tannin, protein, volatile oils, and acids, have been made.

Of late years there has been considerable speculation on the probability that the concentration of free hydrogen-ions in the cell sap of the host is the determining factor in resistance. So far, no definite relation has been established between this character and degree of resistance to parasites. Hawkins and Harvey (14), working on potato varieties resistant and susceptible to *Pythium debaryanum*, Weiss and Harvey (33) on *Chrysophyctis endobiotica* in potatoes, and Priesterbach<sup>4</sup> on wheat rust, all report that hydrogen-ion concentration bears no relation to ability to resist disease. The present investigation was undertaken for the purpose of furnishing additional data on the hydrogen-ion concentration of the expressed juice of a number of wheat varieties, some resistant and others susceptible to stemrust (*Puccinia graminis tritici* Pers.).

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<sup>2</sup> Grateful acknowledgement is here made to Dr. H. B. Humphrey and Dr. H. Hasselbring for their helpful suggestions and criticisms. To the office of Physiological and Fermentation Investigations I am indebted for the loan of equipment.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," 384-386.

<sup>4</sup> SPRIESTERBACH, D. O. BIOCHEMICAL STUDY OF RESISTANCE TO DISEASE IN PLANTS. Unpublished report referred to by Gortner (10, p. 40).



MATERIAL,<sup>5</sup> APPARATUS, AND METHODS

Most of the plants were grown in the greenhouse; but when very young material was desired the seedlings were grown to a height of 3 or 4 inches, without light, in the germinating chambers of the Seed-Testing Laboratory of the Bureau of Plant Industry. By this means, the possibility of error from soil adhering to the plants was avoided. The greenhouse plants were grown either on the benches or in pots; the germinator seedlings on clean cloths on trays in the practically saturated atmosphere of the incubators at a temperature of approximately 20° C. The varieties were always grown and studied in pairs, consisting of a resistant and a susceptible one, as similar morphologically as possible, or in groups of three or four, in order that varying environmental conditions might affect in like degree the resistant and susceptible types in any given set of determinations and thus not obscure inherited differences.

For analysis, germinator seedlings were cut off just above the seed and soil-grown seedlings 1 or 2 inches from the ground in order to avoid contamination by soil that might be clinging to the lower part of the culms. They were ground to a fine pulp in a food chopper which, being without any sharp cutting edges, macerated the material thoroughly. The juice was then squeezed from this pulp by hand through small muslin bags which had been washed and boiled thoroughly in distilled water and used but once. The juice was kept covered in glass-stoppered weighing bottles, and the determinations were made as quickly as possible in order to avoid excessive change in reaction due to oxidations. Centrifuging was not necessary.

The plants were not frozen, as recommended by Dixon and Atkins (8), nor otherwise treated to make the cells permeable before expressing the sap. Harvey (12) has shown that freezing changes the reaction of cabbage juice. It is true that André (1, 2) found that the concentration of the juice of untreated tissue varies inversely with the pressure applied, and that Marie and Gatin (18), Dixon and Atkins (8), and others have shown that the first sample pressed out may be more concentrated than subsequent ones. But Harvey (13) points out that in a buffered plant juice minor differences in concentration do not change the hydrogen-ion measurements appreciably.

The hydrogen-ion concentrations were determined electrometrically. Potential differences were measured with a high-resistance Wolff potentiometer which could be read to 0.00001 volt. The accessory equipment consisted of a Siemens and Halske galvanometer, a Weston standard cell standardized by the Bureau of Standards, and a 2-volt storage battery in series with a Wolff resistance box as the external regulating resistance. The hydrogen electrode usually attained equilibrium in the wheat juice in 10 or 15 minutes. The final reading was taken when the potential difference had not changed by more than 0.0002 volt in five minutes. The saturated calomel electrodes<sup>6</sup> used were made with the usual precautions and frequently checked by the potential difference obtained with the hydrogen electrode immersed in a *M/20* potassium acid phthalate solution,  $P_H$  3.97 (6).

<sup>5</sup> The seed for these experiments was obtained from the agricultural experiment stations at Manhattan, Kans., Akron, Colo., Moccasin, Mont., Hays, Kans., Highmore, S. Dak., St. Paul, Minn., Pullman, Wash., Moro, Oreg., Dickinson, N. Dak., and from the agronomic division of the Office of Cereal Investigations, Bureau of Plant Industry.

<sup>6</sup> The form of these cells was designed by Dr. R. B. Harvey.

The hydrogen electrode was a piece of No. 24 platinum wire 18 mm. long, sealed in the end of a glass tube filled with mercury. The coating of platinum black was deposited in approximately 30 seconds from a 3 per cent solution of platinum chlorid containing a trace of lead acetate. A freshly platinized electrode was used for each determination.

The hydrogen electrode vessel was a stationary, closed cylinder of 30-cc. maximum capacity, the liquid being thoroughly agitated by the hydrogen bubbling through it at the rate of two or three bubbles per second. Electrolytic hydrogen, freed from oxygen by being passed over a palladium asbestos heating coil, was used. It entered the electrode vessel through a side arm of the glass tube enclosing and supporting the hydrogen electrode, this tube being narrowed at the lower end for the length of the platinum wire constituting the electrode so that the bubbles of hydrogen washed down forcibly and broke over the end of the wire.

Most of the measurements were made at a temperature of 25° C. in a small double-walled room, equipped with pipes with circulating water for cooling, a fan, and a heating coil automatically controlled by a Harvey thermoregulator. Temperature changes were noted and taken into account in making the computations.

For calculating the  $P_H$  values from the potential differences measured Michaelis' (20, p. 157-158) values for the saturated calomel cell and for the temperature coefficient were used. The presentation and comparison of data are facilitated by using the simple  $P_H$  units of Sørensen (25) rather than the numbers indicating the normality of the solutions in terms of grams of hydrogen per liter. Throughout this work the procedure was to make at least three successive determinations of each sample, and the average of these determinations was taken to represent the reaction of the juice. Measurements on the same sample practically always agreed within 0.02  $P_H$ .

## RESULTS

### HYDROGEN-ION CONCENTRATION AND VARIETAL RESISTANCE

Table I is a condensed summary of the results of the hydrogen-ion concentration determinations of the expressed juice of resistant and of susceptible varieties at various stages of development, and on which the varying environmental conditions acted in equal degree. Each  $P_H$  value given is an average of the reactions of at least four varieties.

TABLE I.—Hydrogen-ion concentration, in  $P_H$  units, of the expressed juice of wheat varieties in different stages of development and grown in different environments

Environment.	Age.	Averages of resistant varieties. <sup>a</sup>	Averages of susceptible varieties.	Approximate soil reaction. <sup>b</sup>
	Weeks.	$P_H$	$P_H$	$P_H$
Germinating chambers.....	1	5.96 (14)	5.98 (15)	.....
Greenhouse bench.....	1	5.89 (7)	5.92 (7)	7.0
Do.....	2-4	5.86 (14)	5.84 (14)	7.0
Greenhouse pots (unlimed).....	4-6	5.89 (11)	5.87 (5)	7.0
Greenhouse pots (limed).....	4-5	6.13 (7)	6.14 (4)	7.5
Field.....	6-7	5.54 (7)	5.54 (4)	7.0

<sup>a</sup> The figures in parentheses indicate the number of samples averaged.

<sup>b</sup> Determined colorimetrically with Wherry's (34) set of indicators and a set of buffered color standards, for the use of which the writer is indebted to Miss Agnes Quirk, of the Laboratory of Plant Pathology, Bureau of Plant Industry.

The most evident fact revealed by the data is that there is no significant difference in the  $P_H$  values of the juice of resistant and of susceptible varieties of wheat. It is rather surprising that there is so little difference between the reactions of plants of varieties such as Kanred, Little Club, and Preston wheats, and Khapli emmer, representing not only the extremes of susceptibility and resistance to rust but also extremes of morphological characteristics. Another striking fact is that there is little variation in the reaction of the juice of the plant at different stages of growth. The averages show a slightly increased acidity of the greenhouse seedlings 2 to 4 weeks old over that of the same plants 1 week old, and but a small further increase as the plant matures. Environmental factors produce changes in reaction far greater than any due to age or variety.

Table II gives the detailed results by varieties, the grouping showing how they were paired for close comparison. Extra care was taken to grow the plants constituting each group at the same time and under identical conditions. The necessity for such precaution is illustrated by the data on germinator seedlings, which show appreciable differences between certain groups. These are not in any case to be taken as indications of varietal differences, but as indications of the effects of small differences in the conditions under which the groups were grown.

TABLE II.—Hydrogen-ion concentration, in  $P_H$  units, of the expressed juice of some wheat varieties resistant and susceptible to stemrust

Variety.			Age and place of growth of seedlings.					
Name.	C. I. No.	Resistant or susceptible. <sup>a</sup>	7 to 10 days in germinators.	1 to 7 weeks in greenhouse.	4 weeks in greenhouse, unlimed soil	4 weeks in greenhouse, limed soil.	3 months in greenhouse, limed soil.	6 to 8 weeks in field, poor growth.
Kanred.....	5146	R	6.01	5.87				
Turkey.....	1558	S	6.01	5.84	5.87	6.16		
Kanred.....	5146	R	5.95	5.84				
Kharkov.....	1442	S	5.93	5.84				
Pentad (D-5).....	3322	R	5.99	5.87	5.81	6.08	5.88	5.61
Little Club.....	4066	S	5.92	5.87	5.77	6.18		
Khapli.....	4013	R	5.96	5.81	5.81	6.16	5.74	5.40
Kota.....	5878	R	6.05	5.83	5.77	6.17	5.83	5.59
Preston.....	3081	S	6.08	5.79	5.72	6.12	5.76	5.55
Mindum.....	5296	R	5.98	5.88		6.07	5.81	5.59
Arnautka.....	4064	S	5.98	5.87		6.11	5.75	5.55
Iumillo.....	1736	R		5.93	5.93	6.13	5.72	5.54
Average.....			5.99	5.85	5.81	6.13	5.78	5.55

<sup>a</sup> R=resistant; S=susceptible. See column 3 of Table III.

The remarkable agreement between the values obtained for varieties representing widely varying morphological types is strikingly brought out in Table II. Consideration of the figures for any group in any column, and of the results as a whole, leads to the conclusion that varietal resist-

ance to stem rust is not determined by the hydrogen-ion concentration of the cell sap.

Included in the list of wheats of which the hydrogen-ion concentration of the juices is given in Table II are varieties resistant and susceptible to diseases other than stem rust. From data kindly furnished by Dr. E. B. Mains on leaf rust, Mr. C. W. Hungerford on stripe rust, Dr. E. C. Stakman on stem rust, Dr. E. F. Gaines on stinking smut, Dr. W. H. Tisdale and Miss M. A. Griffiths on flag smut, Dr. J. G. Dickson (winter wheats) and Mr. J. J. Christensen (summer wheats) on scab, and Mr. R. W. Leukel on nematodes, together with the results of personal observations on mildew, Table III was compiled.

TABLE III.—Resistance and susceptibility of wheat varieties to various diseases <sup>a</sup>

Variety.	C. I. No.	Stem rust ( <i>Puccinia graminis tritici</i> ).	Leaf rust ( <i>Puccinia tritica</i> ).		Stripe rust ( <i>Puccinia glumarum tritici</i> ).		Stinking smut ( <i>Tilletia tritici</i> ).	Flag smut ( <i>Urocystis tritici</i> ).	Scab ( <i>Gibberella saubinetii</i> ).	Mildew ( <i>Erysiphe graminis</i> ).	Nematode ( <i>Tylenchus tritici</i> ).
		Field and greenhouse.	Field.	Greenhouse.	Field.	Greenhouse.	Field.	Field and greenhouse.	Field and greenhouse.	Field and greenhouse.	Field.
Kanred.....	5146	R*	R	S	S	S	R—	R	S	S	R
Turkey.....	1558	S*	S	—	R—	S+	R	R	S	S	S
Khar'kov.....	1442	S*	R—	—	R	R—	R	—	S	—	—
Pentad (D-s)...	3322	R*	R	—	R	—	R	—	S	—	—
Little Club....	4066	S+	S+	S+	S	S+	S+	—	—	S+	S
Khapli.....	4013	R	R	R*	R—	R	R	S+	—	S	—
Kala.....	5678	R*	S+	S+	—	—	S+	—	R	S	S
Preston.....	3081	S*	S	S	R—	R	R—	—	R	S	S
Mindum.....	5296	R*	R	S*	S	—	R—	—	S	R—	—
Arnautka.....	4064	S*	R	S*	S	S+	R—	—	—	R—	S
Imillo.....	1736	R*	R	S*	S	S	—	—	—	S	—

<sup>a</sup> R = resistant; R— = only moderately resistant; S = susceptible; S+ = extremely susceptible; \* = not susceptible nor resistant to all biologic forms.

The variable behavior of these varieties with respect to infection by the different organisms is interesting, especially since Vavilov (31) has come to the conclusion that if a variety is susceptible or resistant to one it is very likely to be correspondingly susceptible or resistant to all. The uniformly high susceptibility of Little Club and resistance of Khapli are the only examples in the preceding table of consistent behavior in this regard.

From the data given in Table II and Table III, we can conclude that there is no correlation between the hydrogen-ion concentration of the expressed juice and varietal resistance or susceptibility to any of these diseases. The possibility that the total acidity of the cell sap may play an important rôle in the progress of the chemical reactions between the host protoplasm and the destructive secretions of the invading fungus remains and is now being investigated; but that the concentration of free hydrogen ions is a determining factor is precluded by the data here reported.

#### EFFECTS OF ENVIRONMENTAL FACTORS AND EXPERIMENTAL PROCEDURE ON THE HYDROGEN-ION CONCENTRATION OF WHEAT JUICE

In the following pages are described a number of experiments designed to determine the magnitude of errors that are likely to be introduced into measurements of the hydrogen-ion concentration of plant juices,

either as a result of a summation of accidental errors or as a result of systematic errors in operation.

Table IV shows the results obtained from nine different plantings of seed of four varieties, each figure being an average of three determinations. They were grown at different times and so represent effects of the small variations in temperature, moisture, or other environmental conditions in the incubators, which often were sufficient to cause a noticeable difference in the rate of growth of the seedlings. Of course, the handling of the samples from the time the plants were cut until the reaction of the juice was determined was made as uniform as possible.

TABLE IV.—Range in the  $P_H$  values, under the conditions of the experiments, of some wheat varieties resistant, and some susceptible, to stem rust

Experiment No.	Kanred (resistant).	Turkey (susceptible).	Khapli (resistant).	Little Club (susceptible).
1.....	6.06	6.06	5.92	5.92
2.....	6.02	6.00	5.99	5.86
3.....	6.02	6.00	6.00	5.91
4.....	6.06	5.96	5.94	5.92
5.....	5.94	6.00	5.96	5.93
6.....	6.00	5.95	6.03	5.98
7.....	5.99	6.07	6.00	5.92
8.....	5.94	6.04	5.97	5.90
9.....	6.00	5.97	5.94	5.92
Average.....	6.00 ± 0.010	6.01 ± 0.010	5.97 ± 0.008	5.92 ± 0.007

There is a difference of a little more than 0.1  $P_H$  between the extremes for each variety.<sup>7</sup> These are typical of the variations in the values obtained for any variety whether grown in the germinators or in the greenhouse.

In order to determine whether minor differences in procedure during the handling of the sample played any part in producing the range of values reported in Table IV, or whether it was due solely to the slight changes in environmental conditions, four trays of Kharkov seedlings were grown in the same germinator at the same time, one above the other. On the seventh day these were expressed successively with the following results:

Sample No.	$P_H$
1.....	5.922
2.....	5.921
3.....	5.916
4.....	5.921

The close agreement between these values would seem to indicate that such differences as are recorded in Table IV are to be attributed to varia-

<sup>7</sup> These four varieties are especially interesting from the standpoint of their resistance to stem-rust. So far as we know now, Khapli is very resistant to all biologic forms of this rust and Little Club is very susceptible to them all; Kanred is immune from certain forms to which Turkey is very susceptible. According to Table IV, Little Club has a slightly greater hydrogen-ion concentration than the other varieties, the small probable errors of the averages making the difference seem significant. Whether this difference is constant for this variety when grown under incubator conditions was not determined, as too few seed lots were available. However that may be, it is clear that the averages show no correlation of acidity with rust resistance.

tions in the environmental factors affecting growth rather than to errors in operation. In agreement with this conclusion is the statement of Richards (24) that—

Experimentally considered this close connection of acidity with weather conditions gives an exceedingly small expectation of precisely similar results, even with material from the same plant. . . . Even under apparently like conditions the plants are not constant in acidity.

Therefore, for varietal comparisons, no importance can be attached to differences of 0.1  $P_H$  between single observations when the plants are not grown at the same time and under identical conditions. Since so few determinations can be made in a single day, the necessity for averaging a considerable number of observations, if conclusions are to be drawn from small differences, is evident.

It was found that the hydrogen-ion concentration of expressed wheat juice increases on standing, which is in agreement with results reported by Clevenger (7) and Haas (11) for alfalfa and red-clover juice. Some figures showing the rate at which this gradual increase in hydrogen ions takes place in expressed wheat juice, kept in closed weighing bottles at a temperature of 25° C., may be of interest. Three separate determinations were made on the same sample at intervals during the day as indicated below.

A. From plants (Iumillo) 31 days old, grown in limed soil in greenhouse:

a. m.	$P_H$
10.33.....	6.122
10.45.....	6.129
11.00.....	6.132
p. m.	
1.50.....	6.089
2.05.....	6.081
2.15.....	6.071
4.10.....	6.015
4.25.....	5.998
4.35.....	5.996

B. From plants (Kanred) 8 days old, grown in germinating chamber:

a. m.	$P_H$
9.50.....	5.941
10.05.....	5.944
10.20.....	5.939
11.35.....	5.923
11.45.....	5.913
12.00.....	5.913
p. m.	
2.00.....	5.859
2.15.....	5.849
4.00.....	5.813
4.10.....	5.805

These data show that among other precautions, uniform time intervals between the expression of the juice and the determination of its reaction must be observed to avoid errors due to oxidations or other changes in the juice.

Another possible source of error to be investigated was that of dilution. The surface moisture on both greenhouse and germinator plants, especially the latter, varied at different times, and elimination of the resulting slight differences in concentration seemed impossible. Hempel (15) states that a tenfold dilution of the sap of succulent plants produces no essential change in the acidity. Boas (3) found that the hydrogen-

ion concentration of potato juice showed no appreciable change upon the addition of a considerable volume of water. Harvey (13) reports that the addition of one volume of water changed the reaction of the juice of the tobacco plant by only 0.096  $P_H$ . In the present study it was found that, in germinator wheat seedlings a week old, the addition of an equal volume of water increased the  $P_H$  value by 0.06, while the addition of two volumes raised it by 0.10. Greenhouse seedlings 2 to 7 weeks old were less highly buffered against dilution, since with them the addition of one volume of water produced an increase of 0.09  $P_H$  and two volumes, 0.15  $P_H$ . No varietal differences in this respect were found, the determinations being made with the same result with sap from Kanred, Turkey, Khapli, and Little Club. Each figure given is an average of results obtained from six or more different samples. From these determinations we conclude that the small differences in concentration of the juice caused by excess moisture on the leaves, by methods of expressing, or by evaporation during handling do not produce appreciable errors in the hydrogen-ion measurements.

In addition to the errors of manipulation affecting accuracy of measurements, those factors which influence the reaction of the cell sap in the growing plant must be considered before the validity of varietal comparisons can be fully established. It was thought possible that the geographical source of the seed may determine the plant's reactions to new environmental conditions in a sufficient degree to affect its acidity. Whenever possible, resistant varieties and susceptible varieties were obtained from the same experimental farm, but it was seldom possible to obtain Khapli and Little Club, the pair representing the most interesting extremes of resistance and susceptibility, from the same place. It seemed desirable, therefore, to determine the extent of agreement between the concentration of hydrogen-ions in seedlings from seed grown in different places. Below are given the  $P_H$  values of germinator seedlings, a week old, of Khapli emmer from seed obtained from widely separated regions.

Seed from Fullman, Wash., 1921.	Seed from Dickinson, N. Dak., 1921.	Seed from Akron, Colo., 1921.
5.92	6.03	5.94
5.99	6.00	5.96
6.00	5.97	5.94

Three lots of Kanred, two of them from the same station, gave the following results:

Akron, Colo., 1921.	Manhattan, Kans., 1920.	Manhattan, Kans., 1921.
5.99	6.06	6.01
5.93	6.02	5.99
5.94	5.94	5.98
5.95	6.00	5.98
5.94	5.99	5.97

Obviously, geographical differences in the source of the seed have not resulted in sufficient differences in the metabolic processes of the seedlings to affect the hydrogen-ion concentration of the cell sap in a significant degree. It is interesting in this connection to note that Kiessling (17), speaking of the resistance of barley to *Helminthosporium*, states that the specific behavior of varieties was independent of the conditions under which the seed had been grown.

As was to be anticipated from the reported results of previous investigations, it was found that plants cut in the morning almost invariably had a higher hydrogen-ion concentration than those cut from the same plot in the afternoon. As the result of many researches on the diurnal periodicity of the acidity of plant juices, well reviewed and confirmed by Richards (24) and by Clevenger (7), it is a well-established fact that there is a diminution of acid in many plants during the day and a gradual increase during the night until a maximum is reached just before sunrise. Clevenger (7) found that the hydrogen-ion concentration of the cell sap of cowpeas was most acid in the morning, decreasing toward night, and increasing after 9.30 p. m.; and Truog and Meacham (30) reported a corresponding change in the hydrogen-ion concentration of alfalfa. Hempel (15) proved that plants kept in the dark for not too long a time become more acid. In explanation may be mentioned the work of Purjewicz (23), Spoehr (26, 27, 28), and others who have shown that sunlight decomposes malic and other organic acids in vitro. The following figures taken from some of the data obtained during the present investigation, illustrate the magnitude of the difference in reaction between greenhouse wheat seedlings cut in the morning and those cut from the same plots in the afternoon of the same day, all other conditions being kept as nearly identical as possible.

*A. Seedlings 2 weeks old, limed soil*

	Cut 9 a. m.	Cut 1 p. m.
	<i>P<sub>H</sub></i>	<i>P<sub>H</sub></i>
Khapli.....	(a) 5.94 (b) 5.92	5.97
Little Club.....	(a) 6.00 (b) 5.97	6.04
Kanred.....	6.04	(a) 6.13 (b) 6.12
Turkey.....	6.08	(a) 6.16 (b) 6.13
Average.....	5.99	6.09

*B. Seedlings 5 weeks old, unlimed soil*

	Cut 9 a. m.	Cut 1 p. m.
	<i>P<sub>H</sub></i>	<i>P<sub>H</sub></i>
Khapli.....	5.79	5.85
Little Club.....	5.73	5.80
Turkey.....	5.85	5.99
W-5.....	5.79	5.82
Amillo.....	5.82	5.99
Average.....	5.79	5.89



These data show that there is a decrease in the hydrogen-ion concentration of the cell sap between the hours of 9 a. m. and 1 p. m. This changed balance in the reactions involved in acid synthesis and decomposition is not surprising in view of the fact that they have been shown to be so dependent on light, temperature, and moisture conditions (24).

The effect of soil reaction on the hydrogen-ion concentration of the cell sap of plants is not always the same, judging from the observations reported in the literature. Contrary to what one would expect, Clevenger (7) found that the leaves of oats, soy beans, and cowpeas were more acid when there was lime in the soil, although the reverse was true in buckwheat. On the other hand, Kappen and Zapfe (16) state that liming the soil had no effect on the hydrogen-ion concentration of the lupine or bush bean, measured at the time of blooming; and, likewise, Promsy (22) says that the absorption of acids by seedlings did not change the acidity of the sap. Truog and Meacham (30) found, however, that in 12 out of 16 cases, including several different crop plants, lime lowered the reaction of the cell sap. Most of the evidence Haas (11), obtained with many different agricultural plants, indicates that plants grown in unlimed soil have a higher hydrogen-ion concentration than those from limed soil, among these being wheat, although he found a number of plants in which the reverse was true.

All the data bearing on this question of the effect of soil reaction on cell-sap reaction obtained in the present investigation, summed up in the foregoing figures and in Tables I and II, have been consistent in showing a lower hydrogen-ion concentration and lower total acid content of the juice of wheat from limed soil as compared to that from unlimed soil. However, the acidity of the limed seedlings seems to have increased to that of the unlimed seedlings by the time the plants were three months old (Table II). The data in these tables show the degree of difference in the  $P_H$  values and indicate the necessity of eliminating the soil variable in any search for heritable varietal differences in cell-sap acidity.

Whether the greater alkalinity of plants in limed soils is directly due to the absorption of neutralizing ions from the soil, or whether it is the result of some general effect on the health and vigor of the plants, is an open question. In at least one case it was noticed that the limed plants were taller and more vigorous than the unlimed ones with lower  $P_H$ . Clevenger (7) suggested that the greater acidity of the leaves of his limed plants might be explained by assuming that the plants were healthier and metabolism more rapid, but observations on wheat made during the present investigation would indicate that the less vigorous plants are the more acid. Lack of vigor due to unfavorable conditions for growth has been invariably associated with high hydrogen-ion concentration and high total acidity. The most notable examples were winter wheats grown in the greenhouse when the temperature was too high and which had the very low  $P_H$  value of 5.5. Some 14-weeks old Kanred and Turkey wheats, stunted and drooping, were 5.48 and 5.50, respectively, while Mindum and Arnautka growing alongside, heading, and in erect, vigorous growth, were 5.98 and 6.02. When, in other series, these four varieties were equally healthy in appearance, no such differences were found. It was also found that plantings of any variety on a certain shaded bench in the greenhouse resulted in slow-growing plants with lower  $P_H$  values than those from the other benches. It is very interesting to note that Truog and Meacham (30) raise the question as to whether the more acid reaction of lupine in limed soil might not be

due to the fact that liming sometimes injures this species. Also, some irregular results obtained by Haas (11) with plants grown in Plainsfield sand, in which the growth of some plants was found to be unfavorably affected by lime, might well be due to the fact that poor growth is associated with acid accumulation.

In the last column of Table II are given the  $P_H$  values of a number of varieties grown in the virgin soil of a cleared plot in a woodland (Cabin John, Md.). The weather during their growth was very unfavorable, being warm and humid, and the plants when 6 weeks old were far from vigorous. The winter varieties, Kanred, Turkey, and Kharkov, grew so poorly and were so badly infected by *Erysiphe* that no determinations were made on them. The high acidity of these samples was very interesting because the  $P_H$  values agreed closely with the results obtained from unhealthy greenhouse plants. It is interesting to note that Spriesterbach (unpublished report) found that frost caused a definite increase in the hydrogen-ion concentration of wheat plants.

Fungous infection, often present in these cases, was mostly mildew and leaf rust, but the acidity measurements bore no relation, as a rule, to the presence or the degree of infection, so that the acid accumulation was attributed usually to physiological derangements of some of the metabolic processes. Wagner (32), however, claims that the diminution of hydrogen-ion concentration immediately following the injection of phytopathogenic bacteria is followed by a steadily increasing acidity until the end of the incubation period, falling back to normal if the plant successfully resists infection. Mazé (19) says that fungi are active destroyers of organic acid, and Boas (3) says that the acidity of potatoes affected by the leaf-roll disease is less than that of healthy plants. However, Harvey (13) found that the hydrogen-ion concentration of tobacco plants affected with the mosaic disease was somewhat greater than that of the healthy plants, and Weiss and Harvey (33) state that the potato-wart organism caused an increased acidity ( $P_H$  6.00) of the diseased tissue as compared to the healthy tissue ( $P_H$  6.49).

In some of the writer's sowings it was found that wheat plants badly infected with *Erysiphe graminis* were abnormally acid, and it seemed possible that pathological trouble was causal. This was quite clearly indicated by Little Club, which was most susceptible to *Erysiphe*, yet which grew vigorously at greenhouse temperatures when free from disease. In one series, in which Kota, Preston, Pentad (D-5), Mindum, Arnautka, and Khapli, apparently clean, had  $P_H$  values averaging 5.80, Little Club, Kanred, and Turkey, badly infected, had  $P_H$  values of 5.63, 5.64, and 5.67, respectively. In such cases, however, it is impossible to say that the acid accumulation was directly due to the activities of the fungus, as it may well have been the result of decreased vigor attendant upon infection.

#### CONCLUSIONS

- (1) It appears from the data presented in this paper that there is no correlation between the hydrogen-ion concentration of the expressed juice and the resistance or susceptibility of wheat varieties to disease.
- (2) Environmental factors produce much greater differences in the hydrogen-ion concentration of the expressed juice than were ever found between varieties or between plants of different ages grown under identical conditions.

(3) The  $P_H$  value of the juice of wheat plants grown in the greenhouse averages 0.1 higher when the plants are cut at 1 p. m. than when they are cut in the morning about 9 o'clock.

(4) The hydrogen-ion concentration of the juice of wheat plants grown in limed soil is lower than that of plants grown in unlimed soil.

(5) Lack of vigor as shown by unhealthy appearance of wheat plants is always accompanied by an abnormally high acidity of the expressed juice.

(6) Plants badly infected with *Erysiphe graminis* are more acid than adjacent plants free from infection. This may be due to the poor physical condition of the plants and not to any direct effect of the presence of the fungus.

(7) The geographic source of the seed does not affect the hydrogen-ion concentration of the juice of the plants.

(8) The concentration of hydrogen ions in expressed wheat juice increases on standing.

(9) Dilution of the expressed juice decreases the concentration of hydrogen ions, the addition of two volumes of water to one of juice increasing the  $P_H$  value by 0.10 to 0.15. Young germinator seedlings are more highly buffered against dilution than older greenhouse plants.

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# COTTON-WILT, A SEED-BORNE DISEASE<sup>1</sup>

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## INTRODUCTION

The possibility that cotton-wilt is spread by means of infected seed has been recognized for many years, but in the previously reported experiments conducted for the purpose of determining whether or not the disease was seed borne negative results were secured. Fulton<sup>3</sup> discussed the possibility of the disease being carried to new localities on seed but reported that—

The writer has made numerous attempts to secure cultures of the wilt fungus from seed taken from badly wilted plants but without success.

Gilbert<sup>4</sup> later attempted to introduce the wilt into uninfected soil by means of seed obtained from wilted plants. Apparent negative results were obtained in a test covering four successive years on the same piece of ground.

While working with some of the other cotton diseases in Arkansas, the writer was struck by the not infrequent occurrence of isolated wilted plants in otherwise healthy fields, and often on practically virgin land. It was often suggested by the farmers that the disease was introduced in the seed. The possibility of the disease being seed-borne is very evident to one familiar with the disease and the methods of cotton planting. As with most wilt diseases, plants die throughout the season and some live through to frost. The most severely attacked plants, or those infected earliest, die without producing mature bolls. Many wilted plants, however, produce mature bolls before dying. Usually the plant dies, leaving many bolls in various stages of maturity, some of which open or partly open during the picking season. During a period of wet weather the wilt fungus grows out to the surface of the dead terminal twigs of the diseased plants and often covers the surface with a coating of spores. Under such conditions it is hard to conceive how any of the "seed cotton" from a badly wilt-infected field could escape carrying the spores of the fungus on the lint.

In the spring of 1920 over 200 bushels of cotton seed were delinted by the concentrated sulphuric-acid method and further disinfected by soaking in 1 to 1,000 corrosive sublimate solution. Most of this seed was planted on virgin timber-land slash in Mississippi County, Ark., at a considerable distance from other cotton land. In this field of nearly 200 acres occasional wilt-infected plants appeared throughout the season. No satisfactory explanation of their occurrence other than that the

<sup>1</sup> Accepted for publication July 17, 1922.

<sup>2</sup> The writer acknowledges the assistance of Mr. R. F. Crawford in carrying on the isolation and inoculation experiments.

<sup>3</sup> FULTON, H. R. COTTON WILT. *La. Agr. Exp. Sta. Bul.* 96, 15 p., illus. 1907.

<sup>4</sup> GILBERT, W. W. COTTON DISEASES AND THEIR CONTROL. *U. S. Dept. Agr. Farmers' Bul.* 1187, 32 p., illus. 1921.

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organism was carried within the seed seemed possible. In 1921 in a small plot of cotton grown at Fayetteville, from thoroughly surface disinfected seed, two wilt plants appeared. The plot was far from the cotton area and had never before been planted to cotton. One of the two diseased plants died early, producing no bolls; the other lived throughout the season. These two cases strongly suggested the possibility that the cotton-wilt fungus may be carried on the inside of the seed coat, and the experiments here reported were directed primarily toward discovering whether or not such was the case.

#### METHODS OF PROCEDURE

Seed was carefully selected from wilt-infected cotton plants, delinted with concentrated sulphuric acid as a preliminary treatment, then further disinfected before planting. The seed was germinated under aseptic conditions, and as far as possible all fungi appearing on either viable or dead seed were identified. Fungi which from their morphology and their biochemical reactions were judged to be *Fusarium vasinfectum* Atk. were saved for further tests expected to establish their identity. No fungus was definitely accepted as being *F. vasinfectum* until successful inoculations of cotton plants had been secured. At first the plants which had died, apparently of wilt, were strongly surface sterilized, and the fungus was reisolated from all parts of the stem; but in the later tests the typical wilting, accompanied by the blackening of the xylem, was considered final proof of the identity of organism as *F. vasinfectum*.

#### ISOLATION OF THE FUNGUS

Seed from badly wilted cotton plants, in which the vascular infection could be traced into the bolls from which the seed was taken, was selected in the field September 21 to 24, 1921. The selections were made with particular care, which fact probably had much to do with the results subsequently obtained. After the longer lint had been hand picked from the seed, the seed was delinted with concentrated sulphuric acid, washed and dried, and put away in a cloth bag until used for plating. Each lot of seed was again subjected to about five minutes' treatment with concentrated sulphuric acid, washed, treated for two minutes in 1 to 1,000 mercuric-chlorid solution, and washed in sterile water before plating. It would seem impossible for any organism external to the seed coat to survive this treatment, and all organisms isolated from seed so treated have been considered as coming from inside the seed coat.

Plating was begun September 27, three days after the seed was gathered. Four series of platings were made within a month of the time the seed was gathered, and the wilt fungus was isolated 31 times from 524 seeds, or from practically 6 per cent of the seeds plated. Within three months from the time the seed was gathered, the fungus had been isolated from 39 of 769 seeds plated. The first plating was made on cornmeal agar, but in all following series the plating was done on white blotting paper in Petri dishes sterilized in an autoclave. All fungi appearing on the seeds were saved for identification, at first by transferring them to corn or potato agar stants, later by transferring the suspected fungi to rice tubes for the color reaction of the fungi, which in the case of the wilt organism is rose pink, or a little darker. Germinated seeds which showed

no indication of disease were transferred to test tubes of corn meal or potato agar, originally with the intention of using them for inoculation tests, but, as several of these seedlings developed signs of wilt, they were saved in later series for the purpose of observing possible development of signs of wilt infection. Nearly half of the total isolations of the wilt fungus were secured by this method (Pl. 2, A).

After the definite identification of some of the fungi isolated from the cotton seed as *Fusarium vasinfectum*, the remainder of the seed was saved for plating at intervals of about one month, for the purpose of determining the length of time the fungus may remain viable within the seed.

The isolation series is given in Table I.

TABLE I.—Isolations of *Fusarium vasinfectum* from cotton seed.

Date isolated.	Series.	Number of seeds.	Number of isolations of <i>F. vasinfectum</i> .	Viable infected seed. <sup>1</sup>	Viable seed not infected. <sup>1</sup>	Dead infected seed. <sup>1</sup>	Dead seed not infected. <sup>1</sup>
Sept. 27, 1921.....	A.....	105	4	2	68	3	46
Sept. 28, 1921.....	X.....	119	5	2	77	1	35
Oct. 6, 1921.....	B.....	150	6	4	109	1	45
Oct. 16, 1921.....	C.....	150	5	4	50	3	42
Oct. 25, 1921.....	D.....	150	5	4	35	1	54
Dec. 12, 1921.....	H.....	95	3	0	80	3	116
Jan. 17, 1922.....	K.....	100	1	0	82	1	117
Feb. 16, 1922.....	J.....	200	4	1			
Mar. 14, 1922.....	Y.....	200	1	0			
Apr. 17, 1922.....	T.....	200	1	0			
Sept. 27 to Oct. 16, 1922.	F <sup>2</sup> .....	11	11	11			
Total.....		1,469	46	22	413	14	458

<sup>1</sup> The isolation of the wilt fungus only is considered here in the terms "infected" and "not infected."

<sup>2</sup> The F series was isolated from what at first were apparently healthy seedlings from the series A, X, B, and C, after these had grown for some time on agar in test tubes.

An examination of Table I will show that the percentage of isolations of *Fusarium vasinfectum* fell off rapidly after the third month. The fungus was recovered 39 times from 769 seeds plated within three months of the date of collecting, and only 7 times from 700 seeds plated from the fourth to the seventh month, the percentage of isolations falling from approximately 6 per cent in the first month to  $\frac{1}{2}$  per cent in the sixth and seventh months. The plating of April 17 exhausted the supply of selected seed. For the first six series of isolations, plump, viable-appearing seed was selected, which left a large percentage of light, immature seeds in the later series and may have affected the results to some extent, although there is no apparent reason why it should, unless the presence of the wilt fungus in the immature seed may have been more often masked by the presence of other fungi.



## INOCULATION EXPERIMENTS

As has been stated above, positive inoculation results were made the final proof of the identity of the isolated organisms as *Fusarium vasinfectum* Atk. Fusaria which from their morphology were judged to be the wilt organism were used to inoculate pots of soil sterilized in an autoclave. Corn meal or cottonseed meal cultures were used in the earlier tests. Also, uninoculated meal was put in the control pots, but the meal made so favorable a medium for fungous and bacterial growth that plants in both the control and inoculated pots were killed by almost any organism which chanced to infect the meal. The use of even small quantities of corn or cottonseed meal gave these results. The toxicity to cotton seedlings of the banana-wilt fungus as reported by Brandes<sup>1</sup> can probably be explained as a similar case of "damping-off." This difficulty was avoided by the use of sterilized cotton stem fragments carrying the inoculum, the infected material being placed under the surface layer of soil at the time the seed was planted. Apparently healthy acid-delinted seed was used for all plantings.

The seedlings growing in soil infected with the wilt organism in some instances began to show the effects of inoculation in eight days after planting, while occasional plants lived for several weeks or months after the other plants growing in the same pots had died of wilt. There was apparently much variation in the virulence of different cultures isolated, judging from the behavior of the plants in different pots in the same series of inoculations. If the seedlings did not wilt soon after germination, they usually survived for a considerable time before showing signs of infection. No culture which had not been judged from its morphology to be of the wilt organism ever caused an infection resembling wilt, although all Fusaria obtained among the earlier isolations were tested for possible pathogenicity. The color reaction of the wilt fungus on rice also came to be considered an almost positive indication of its identity, and, in later series, no cultures were used in infection experiments which did not give the characteristic color reaction. While at first the organism was reisolated from all parts of the wilted plants, in later inoculations the wilting of the plants, coupled with the typical blackening of the xylem, was considered sufficient proof of the identity of the organism as *Fusarium vasinfectum* (Pl. 1, A). In the earlier series both uninoculated pots and pots inoculated with known wilt cultures were run as controls, but in the later series the known wilt controls were omitted. Table II summarizes the inoculation experiments.

An examination of Table II will reveal a great variation in the length of time elapsing between the planting of the seeds in the inoculated soil and the isolation of the fungus from infected plants. This may have been due to various causes. In some cases the plants died without showing positive indications of wilt but merely rotted off at the soil line. During the winter months the growing conditions were very unsatisfactory, because of poor greenhouse facilities. Apparently there was a great variation both in the virulence of the cultures and in the resistance of plants. Practically every culture has been tried two or three times, and some have been consistently highly destructive while others have been less so. In some cases a plant or two survived a long time

<sup>1</sup> BRANDES, E. W. BANANA WILT. In *Phytopathology*, v. 9, p. 339-389, 5 figs., pl. 21-34 (partly col.) 1919. Literature cited, p. 389-389.

after the others had died, as in the case of the culture D 9. The pot was inoculated on November 28, the first reisolation of the wilt fungus from a wilted plant was made February 5, and the last plant shed all its leaves on March 18. This plant had a blackened xylem from the roots to the growing point of the plant (Pl. 1, B). Some of the other cultures produced rather mild cases of wilt in plants which grew for several months.

TABLE II.—Inoculation experiments with *Fusarium vasinfectum* isolated from cotton seed.

Date planted.	Series No.	Wilt reisolated.	Date planted.	Series No.	Wilt reisolated.
Nov. 28, 1921	A 9.....	Feb. 21, 1922	Mar. 13, 1922	H 13.....	Apr. 27, 1922
Do.....	B 1.....	Dec. 15, 1921	.....do.....	K 1.....	Apr. 22, 1922
Do.....	B 2.....	Dec. 23, 1921	Mar. 29, 1922	J 1.....	June 1, 1922
Do.....	B 4.....	Feb. 23, 1922	.....do.....	J 2.....	June 3, 1922
Do.....	B 9.....	Apr. 18, 1922	.....do.....	J 3.....	June 1, 1922
Do.....	C 2.....	Jan. 23, 1922	Apr. 24, 1922	Y 1.....	June 3, 1922
Do.....	C 4.....	Feb. 5, 1922	May 22, 1922	A 10.....	June 1, 1922
Do.....	C 5.....	Dec. 15, 1921	.....do.....	B 6.....	June 3, 1922
Do.....	C 10.....	Dec. 17, 1921	.....do.....	B 11.....	May 30, 1922
Do.....	C 12.....	Feb. 13, 1922	.....do.....	D 4.....	( <sup>1</sup> )
Do.....	D 7.....	Mar. 23, 1922	.....do.....	D 6.....	( <sup>2</sup> )
Do.....	D 9.....	Feb. 5, 1922	.....do.....	D 15.....	June 10, 1922
Do.....	F 1.....	Feb. 13, 1922	.....do.....	F 3.....	( <sup>2</sup> )
Do.....	F 2.....	Dec. 17, 1921	.....do.....	F 4.....	June 10, 1922
Do.....	F 5.....	Dec. 15, 1921	.....do.....	F 8.....	Do.
Do.....	F 6.....	May 18, 1922	.....do.....	F 9.....	June 16, 1922
Do.....	F 7.....	Dec. 30, 1921	.....do.....	F 10.....	( <sup>2</sup> )
Do.....	X 1.....	Dec. 23, 1921	.....do.....	F 11.....	June 5, 1922
Do.....	X 3.....	Dec. 15, 1921	.....do.....	F 12.....	June 7, 1922
Do.....	X 4.....	.....do.....	.....do.....	F 13.....	June 12, 1922
Do.....	X 5.....	Dec. 17, 1921	.....do.....	X 2.....	( <sup>2</sup> )
Mar. 13, 1922	H 6.....	June 3, 1922	.....do.....	J 4.....	May 31, 1922
Do.....	H 8.....	Apr. 27, 1922	.....do.....	T 1.....	June 5, 1922

<sup>1</sup> Pot destroyed.

<sup>2</sup> These cultures had not produced wilt up to Oct., 1922.

#### LONGEVITY OF THE WILT FUNGUS BORNE EXTERNALLY ON COTTON SEED

The isolation of the wilt fungus from surface-sterilized seed plated April 17 and its successful inoculation on cotton seedlings proved quite definitely that the cotton-wilt fungus can live over from one season to the next within the cotton seed. Another point of interest was the length of time the fungus spores would remain viable on the outside of the seed. In an effort to determine this point, on January 2 a considerable quantity of undelinted seed was quite thoroughly infected by mixing it with a large culture of the known wilt organism growing on inoculated cotton stem fragments. After thorough mixing to secure a good distribution of the spores the stem fragments were picked out. This seed was first air dried, then put away in a covered battery jar. From 50 to 100 of these seeds were plated out each month up to May 1. At every plating from 80 to 100 per cent of the seed yielded viable spores of the wilt organism. The identity of the organism isolated from this seed was determined by its morphology and color reaction and in no case by inoculations. The later platings have been made

on white blotting paper which before sterilizing was dipped in a rice-starch solution so that the color reaction of the fungus may be observed as soon as the growth of the fungus begins. The color produced on the rice-starch paper is much darker purple, more like the iodine starch reaction, than that produced on rice alone, but it is quite constant and was considered dependable in this test.

On May 3 infected seed was planted in three short rows in soil which had never before been in cotton. About 1,000 seeds were planted, and the germination was good. On May 27 a dozen or more of the seedlings from this planting were found dying and thoroughly infected with wilt. On June 9 the plants were thinned to two or three in a hill, leaving 378 plants in the plot. All plants removed were examined, by sectioning, for signs of wilt. Of the 493 plants examined, 32 gave evidence of wilt infection, making about 5 per cent of the plants showing infection up to that time.

How long the spores on the seed will remain viable in this test still remains to be determined, but it is very evident that it will live the usual period between picking time and planting. It is probable that the number of spores occurring on the artificially inoculated seed is considerably greater than is likely to occur naturally, but Edgerton<sup>6</sup> has shown that the number of spores of various fungi occurring naturally on cotton seed may be enormous. Under favorable conditions the number of *Fusarium vasinfectum* spores could easily be as high as reported by Edgerton for other fungi, and, under the ordinary bin storage of cotton seed, at least a considerable number should remain viable until planting time.

It would seem likely that spores of the wilt fungus might be introduced into a field without infecting the soil, or at least that several years might elapse after its introduction before the infection would become noticeable. In the light of the results obtained in the writer's work, it would seem that some such explanation must be necessary to account for Gilbert's<sup>7</sup> apparent negative results. In the planting of artificially infected seed mentioned above, at least 80 per cent and probably 100 per cent of the seed carried the wilt organism at the time of planting, yet only a very few plants died of wilt in the early seedling stage and those could easily have been overlooked if the plot had not been examined carefully. At the time of thinning, also, only a few of the plants having wilt showed a general infection, and many of those infected might not have given much external evidence of the disease if they had remained in the field. Undoubtedly those seeds which carry the wilt internally stand a poor chance of producing a plant that will live more than a very short time, and it is probable that an introduction of the wilt by such seed would escape notice the first year unless secondary infection of adjoining plants should take place.

It would seem that under the present system of saving cotton seed and the planting of very large quantities of seed to insure a stand, every step in cotton planting favors the introduction of the wilt disease into new fields and its general distribution in all fields. The results of the present work would seem to justify the recommendation that seed for planting purposes shall not be saved from fields badly infected with wilt. Acid

<sup>6</sup> EDGERTON, C. W. THE ROTS OF THE COTTON BOLL. La. Agr. Exp. Sta. Bul. 137, 113 p., 13 pl. (in text). 1912. Bibliography, p. 81-85.

<sup>7</sup> GILBERT, W. W. COTTON DISEASES AND THEIR CONTROL. U. S. Dept. Agr. Farmers' Bul. 1187, 31 p., 18 fig. 1921.

delinting will undoubtedly reduce the chances of introducing the disease, although organisms carried within the seed coat will escape this treatment.

#### SUMMARY

The cotton wilt organism, *Fusarium vasinfectum* Atk., was isolated from strongly surface-sterilized cotton seed, indicating that the organism is at times carried on the inside of the seed coat. The pathogenicity of the organism was proved by inoculation experiments. Artificially inoculated seed carried the viable organism on the seed lint for at least five months. The wilt disease was introduced into wilt-free soil by means of artificially infected seed. It is recommended that badly infected fields be rejected as a source of seed for planting.

PLATE 1.

A.—Sections of healthy cotton plant from control pot on right. Sections of plants from pot inoculated with culture No. H 8 on left.

B.—Plants above in uninoculated control pots. Plants below in pots inoculated with culture No. B 1, X 3, C 5, X 4, F 5, all dying of wilt.





